Scientific Research Support Fund

The Hashemite Kingdom of Jordan

The University of Jordan

المجلة الأردنية في العلوم الصيدلانية

مجلة علمية عالمية متخصصة تصدر بدعم من صندوق دعم البحث العلمي والابتكار

Jordan Journal of PHARMACEUTICAL Sciences

Specialized International Referreed Journal

Issued by the Scientific Research Support Fund

This title is indexed in Scopus

مجلد (17) العدد (4)، كانون الأول 2024

Volume 17, No. 4, December 2024

Established 2007

ISSN: 1995-7157

EISSN: 2707-6253

Publisher

The University of Jordan
Deanship of Scientific Research
Amman 11942 Jordan
Fax: +962-6-5300815

National Deposit
(23.3/2008/D)
(Journal's National Deposit Number at the Jordanian National Library)

© 2024 DSR Publishers

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means: electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the publisher.

Jordan Journal of Pharmaceutical Sciences

Volume 17, Number (4), *December* 2024

Editor-in-Chief
Prof Ibrahim Alabbadi

Editorial Board

Prof Yusuf Al-Hiari
Prof Mutasim Al-Ghazawi
Prof Wael Abu Dayyih
Prof Tareq Lewis Mukattash
Prof Faisal Tawfiq Al-Akayleh
Prof Reema Abu Khalaf

Advisory Board Members

Prof. Zoltán Kaló
Center for Health Technology Assessment, Semmelweis University, Hungary
Prof. Ahmad Agil Abdalla
Biomedical Institute Research Center, Granada University, Granada, Spain
Prof. Nathorn (Nui) Chaiyakunapruk
University of Utah, USA
Prof. Ryan F. Donnelly
Chair in Pharmaceutical Technology, Queen's University Belfast, UK
Prof. Samir Ahid
Mohammed VI University of Health Sciences, Casablanca, Morocco
Prof. Udo Bakowsky
Philipps University Marburg, Marburg, Germany
Prof. Ayman F. El-Kattan
Executive Director, IFM Management Inc., Boston MA, USA
Prof. Paul Anthony McCarron
Head of School of Pharmacy and Pharmaceutical Sciences, University of Ulster, UK
Prof. Khalid Z Matalka
Matalka's Scientific Writing, Lexington, MA, USA
Prof. Habil. Wolfgang Weigand
Institute for Inorganic Chemistry and Analytical Chemistry, Friedrich Schiller University Jena, Germany
Prof. Ashraf Mostafa Abadi
Head, Pharmaceutical Chemistry Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, Egypt
Prof. Juan Manuel Irache Garreta
Universidad de Navarra, Pamplona, Madrid, Comunidad de, Spain
Prof. Ahmad Telfah
Leibniz Institut für Analytische Wissenschaften, ISAS Bunsen-Kirchhoff Str, German
Prof. Ali Qaisi
Faculty of Pharmacy, The University of Jordan, Amman, Jordan
Prof. Alsayed Alarabi Sallam
Al Taqadom Pharmaceuticals, Amman, Jordan
Prof. Karem Hasan Alzoubi
Faculty of Pharmacy, Jordan University of Science and Technology, Amman, Jordan
Prof. Yasser Bustanji
Faculty of Pharmacy, The University of Jordan, Amman, Jordan
Prof. Mayyas Al Remawi
Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan
Prof. Talal Ahmad Aburjai
Faculty of Pharmacy, The University of Jordan, Amman, Jordan
Prof. Qosay Ali Al-Balas
College of Pharmacy, Jordan University of Science & Technology, Irbid, Jordan

Editorial Secretary
Sana' Al-Dhgely

English Language Editor
Lama Khalifah

Producion
Na'eemeh Mofeed Al-Sarrawi

Jordan Journal of Pharmaceutical Sciences

Aims & Scopes:

***Jordan Journal of Pharmaceutical Sciences* (*JJPS*)** is a bimonthly open-access peer reviewed journal funded by the Scientific Research Fund at Ministry of Higher Education and Research and hosted by the Deanship of Research at the University of Jordan. JJPS is dedicated to various disciplines of pharmaceutical and allied sciences. JJPS publishes manuscripts on original work, either experimental or theoretical in the following areas:

- Pharmaceutics & Biopharmaceutics
- Drug Delivery systems
- Nanotechnology & Nanomedicine
- Organic & Medicinal Chemistry
- Pharmacognosy & Phytochemistry
- Pharmacology, Toxicology, and Experimental Therapeutics
- Pharmaceutical Biotechnology
- Microbiology
- Pharmacy Practice
- Clinical Pharmacy & Hospital Pharmacy
- Pharmacovigilance & Drug Safety
- Health Outcomes and Economics
- Pharmaceutical Health Care Services
- Natural Product Research
- Drug Regulatory Affairs
- Health Policy
- Pharmaceutical Marketing

Instructions to Authors

Preparation and Submission of Manuscripts

Type of Manuscripts

JJPS publishes original research articles, full reviews, research reports, short communications, case studies, commentaries, and short reviews.

Manuscript Preparation

Research paper should be typed on the computer; double spaced, and shouldn't exceed 15 pages (3000 words, font size 12). Spelling, punctuation, sentence structure, spacing, length, and consistency in form and descriptions should be checked before submission. References should also be checked for accuracy. Ensure that all figures and tables are mentioned in the text, and that all references are cited in the text.

Title Page

A separate title page should be submitted separately with the manuscript. The title should be followed by the author(s) name(s) and full affiliations including institution name and address. The title, author(s) name(s), and affiliations should all appear on their own respective line of text. Place an asterisk after the name of the author to whom enquiries regarding the paper should be directed and include that author's telephone and fax numbers and e-mail address. Author(s) affiliation (s) must be mentioned for each one in order.

Abstract

Authors should submit with their research two abstracts, one in English and it should be typed at the beginning of the paper followed by the keywords before the introduction.

The abstract, detailing in one paragraph the problem, experimental approach, major findings, and conclusions, should appear on the second page. It should be double spaced and should not exceed 200 words for **Full Papers and Reviews or l00 words for Case Studies and Short Communications.**

Compounds mentioned in the abstract, given as specific Arabic numbers in the text, should also be accompanied in the abstract by that same numeral. The abstract should appear on a separate page and should be untitled.

Authors are required to submit an Arabic abstract (Required only for Arab native speakers) which should be appended at the end of the submitted manuscript on a separate sheet, including author(s) name(s) and affiliation(s).

Keywords

Should be included at the end of the abstract page, separated by semicolons, not exceeding seven both in Arabic and in English.

Introduction

The manuscript should include an introduction stating the purpose of the research detailed in the manuscript and summaries previous literature leading to the idea and method described in the manuscript.

Results

The results should be presented concisely. Tables and figures should be designed to maximize the presentation and comprehension of the experimental data. Data present in tables and figures should not be redundant in the manuscript's text.

Discussion

The discussion section should interpret the results and relate them to existing knowledge in the field in as clearly and brief as possible. For Full Papers, subheadings may be included within the Results and Discussion sections.

Bolded structural code numbers should only be used for new compounds and for those known compounds for which new biological data or spectroscopic values are being reported. Other known compounds should be referred to in the text by name, wherever necessary.

Experimental Section

The presentation of specific details about instruments used, sources of specialized chemicals and related experimental details should be incorporated into the text of the Experimental Section as a paragraph headed General Experimental.

Acknowledgments

The Acknowledgments section should include credits [initial(s) and last name(s)] for technical assistance, and other appropriate recognition.

Conflict of Interest

Authors should declare any pertaining conflicts in their work, otherwise the authors should clearly indicate that no conflict is present.

Funding

Authors should declare any received funding, indicating the funding institution and grant number. Otherwise, the receipt of no funding should be indicated.

References and Notes

References to the literature and all notes, regardless of their nature, should be numbered in order of appearance in the manuscript, and then cited under the heading References and Notes with superscript numbers. Each reference may have its own citation number, then should be assigned its own number in this section. References and notes should follow the format shown:

Journal

Taha M., Al-Bakri A. and Zalloum W. Discovery of potent inhibitors of pseudomonal quorum sensing via pharmacophore modeling and silico screening. *Bioorg. Med. Chem. Lett.* 2006; 16:5902-5906.

Book

Ancel H. C., Allen L. V. and Popovich N. G. *Pharmaceutical Dosage Forms and Drug Delivery Systems*; Lippicott Williams & Wilkins: New York. 1999, p 45.

Chapter in a Book

Aburjai T., Natsheh F. and Qasem A.: *In: Contemporary Perspective on Clinical Pharmaceutics*. Kohli K. (Ed).; Elsevier New York, 2006; 1st edition, Chapter 57, pp 623-633.

Chemical or Biological Abstract

Al-Hiari Y., Qaisi A., El-Abadelah M. and Wolfgamg V., *Monatshefte fuer Chemei*. 2006; 137(2) 243-248, *Chem. Abstr*. 2007; 145, 397308.

Ph.D. or M. Sc. Thesis

Alkhalil S. The Alkaloids of *Thalictrum isopyroides*. Ph.D. Thesis, Pittsburgh University, PA. 1986, p 115.

Patent

Davis R. U.S. Patent 5,708,591, 1998.

The author is responsible for the accuracy and completeness of all references.

All references must be numbered and written "superscript "without parentheses in the Manuscript (*e.g....according to guidelines pertaining to these techniques*[5,6,7] **);but with parentheses around in the references list (***e.g. (1) Alkhalil S. The Alkaloids of Thalictrum isopyroides. Ph.D. Thesis, Pittsburgh University, PA. 1986, p 115).*

Nomenclature

It is the responsibility of the author(s) to provide correct nomenclature.

Insofar as possible, authors should use systematic names similar to those used by Chemical Abstracts Service.

Abbreviations

Standard abbreviations should be used throughout the manuscript. All nonstandard abbreviations should be kept to a minimum and must be defined in the text following their first use. The preferred forms of some of the more commonly used abbreviations are: mp, bp, °C, K, s, min, h, mL, . ***μ***L, kg, g, mg, ng, ***μ***g, cm, mm, nm, mnl, mmol, , ***μ***mol, ppm, TLC, GC, HPLC, NMR, MS, UV, and IR.

Graphics

The quality of the illustrations printed depends on the quality of the originals provided. Figures cannot be modified or enhanced by the journal production staff. Whenever possible, the graphic files furnished by authors on CD with revised submissions of accepted manuscripts are used in production of the Journal.

A- Layout

In preparing structures for direct photoreproduction, layout is critical. Equations, schemes and blocks of structures are presented in the Journal either in one-column or two-column format.

B- Content

Abbreviations such as Me for CH_3, Et for C_2H_5 and Ph (but not Φ) for C_6H_5 are acceptable.

C- Dimensions

For best results, illustrations should be submitted in the actual size at which they should appear in the Journal. Only original work or high quality photographic prints of originals should be submitted; photocopies do not reproduce well.

Chemical Structures

Structures should be produced with the use of a drawing program such as Chem-Draw. Structure drawing preferences are as follows:

1- Drawing settings:

Chain angle 120°

Bond spacing 18% of width

Fixed length 14.4 pt (0.508 cm, 0.2 in.), Bold width 2.0 pt (0.071 cm, 0.0278 in.), Line width 0.6 pt (0.021 cm, 0.0084 in.), Margin width 1.6 pt (0.056 cm, 0.0222 in.), Hash spacing 2.5 pt (0.088 cm, 0.0347 in.)

2- Text settings:

Font: Arial/Helvetica

Size: 10 pt

3- Preference:

Units: points

Tolerance: 3 pixels

4- Page setup:

Paper: US Letter

Scale: 100%

Tables

These should be numbered consecutively with Arabic numerals and should be arranged within the manuscript.

Footnotes in tables should be given lowercase letter designations and be cited in the table by italic superscript letter.

Figures

Figures should be constructed in keeping with the column width and line width. All illustrations should be numbered as "Figures", with Arabic numerals.

The Arabic numbers (not the roman ones or the alphabets) are used to number the Tables and Figures which are not abbreviated into Fig. or Tab.

Informed Consent

All manuscripts reporting the results of experimental investigation involving human subjects should include a statement confirming that an informed consent was obtained from each subject or subject's guardian, after the approval of the experimental protocol by a local human ethics committee or IRB.

Copyright Status Form

A properly completed Copyright Status Form with an original signature in ink must be provided for each submitted manuscript.

Manuscript Submission

Manuscripts (in English), together with a cover letter from the corresponding author, should be submitted. A valid e-mail address should be listed when submitting manuscripts.

Manuscript submission via **Website**: **https://jjournals.ju.edu.jo/index.php/jjps/**

Galley Proofs

Page proofs will be sent to the author who submitted the paper. The standard delivery method for galley proofs is by mail.

Correspondence

Correspondence regarding accepted papers and proofs should be directed to Jordan Journal of Pharmaceutical Sciences.

Deanship of Scientific Research
The University of Jordan
Amman 11942, Jordan
Phone: +962 6 5355000 Ext. 25106
Fax: 00962 6 5300815
E-mail: jjps@ju.edu.jo
Website: **https://jjournals.ju.edu.jo/index.php/jjps/**

INTRODUCTION

The Jordan Journal of Pharmaceutical Sciences (**JJPS**) is a peer-reviewed Journal, which publishes original research work that contributes significantly to further the scientific knowledge in pharmaceutical sciences' fields including pharmaceutical/medicinal chemistry, drug design and microbiology, biotechnology and industrial pharmacy, instrumental analysis, phytochemistry, biopharmaceutics and Pharmacokinetics, clinical pharmacy and pharmaceutical care, pharmacogenomics, bioinformatics, and also **JJPS** is welcoming submissions in pharmaceutical business domain such as pharmacoeconomics, pharmaceutical marketing, and management. Intellectual property rights for pharmaceuticals, regulations and legislations are also interesting topics welcomed from our colleagues in Schools of Law.

On a current topic in Pharmaceutical Sciences are also considered for publication by the Journal. **JJPS** is indexed in SCOPUS (Q3). It's a journal that publishes 4 issues per year since 2021 in (**March, June, September, December**). The Editorial Team wishes to thank all colleagues who have submitted their work to JJPS). If you have any comments or constructive criticism, please do not hesitate to contact us at jjps@ju.edu.jo. We hope that your comments will help us to constantly develop **JJPS** as it would be appealing to all our readers.

Prof Ibrahim Alabbadi
Editor-in-Chief
School of Pharmacy- The University of Jordan
Amman 11942- Jordan

Volume 17, 2024

Letter from the Editor-in-Chief

Typically, Food and Drug Administration (FDA) organizations or health authorities in countries worldwide perform all necessary investigations before granting approval for any medication or any type of food suitable for human consumption. But what happens when these authorities do not exist? Can we expect peace in 2024? We all hope every human, irrespective of their geographical location, can live in peace and enjoy good health, with all essential life necessities readily available. The World Health Organization's recent definition of health refers to a state of complete physical, social, and mental well-being, NOT ONLY the absence of disease or infirmity. Health-Related Quality of Life is a fundamental right for any human living on earth. But what if there is no food or medication, or if these essentials are even prohibited?

The Jordan Journal of Pharmaceutical Sciences (JJPS) team includes numerous scholar colleagues from universities in Gaza which have been demolished. These colleagues may still be alive or sadly, they may have already lost their lives. Numerous professors supported the JJPS by acting as peer reviewers and submitting quality scientific articles under incredibly challenging circumstances. We tip our hats to all of them and hope that God, with his immediate power, will grant life, peace, and security as Ramadan commences in March.

The JJPS editorial board has already initiated the second phase of a three-year term, following renewal approval from the Jordanian Ministry of Higher Education, which underscores our tremendous teamwork and significant progress. The JJPS' scores in international scientific databases, such as SCOPUS, continue to improve – our Q3 score is now close to Q2. Additionally, we've seen a continued influx of submissions from increasingly diverse countries, including places in North Africa, Europe, the USA, Canada, Australia, and Southeast Asia. We've also noticed a significant reduction in time from submission through revision to the decision-making process, and with the rise in ambiguity due to AI and ChatGPT programs, the need for similarity report checks has become essential.

Best regards

Prof Ibrahim Alabbadi
Editor-in-Chief

CONTENTS

Isolation, Characterization, and Assessment of Probiotic Properties of *Bacillus clausii* Isolated from Children's Stools in a Northern Province of Vietnam

Nguyen Quynh Anh Ngo[1*], Xuan Thanh Dam[2], Tiep Khac Nguyen[2], Chien Ngoc Nguyen[3*], Nhi Dinh Bui[4*]

[1]Center for Pharmaceutical Research and Business, Hai Duong Central College of Pharmacy, 324 Nguyen Luong Bang, Hai Duong City, Vietnam.
[2]Faculty of Biotechnology, Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Ha Noi City, Vietnam.
[3]National Institute of Pharmaceutical Technology, Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Ha Noi City, Vietnam.
[4]Viet Tri University of Industry, Viet Tri City, Vietnam

ABSTRACT

Bacillus clausii is a widely utilized human probiotic in various commercial products; however, there has been limited research on the isolation from diverse sources and evaluation of probiotic characteristics of *Bacillus clausii*. For the first time in this study, *Bacillus clausii* strains were isolated and evaluated from stool samples obtained from healthy volunteer children in a northern province of Vietnam. The inherent biological properties of the isolated *Bacillus clausii* strains were specifically examined to explore their potential application as probiotics. Thirteen colonies underwent screening through morphological and biochemical analyses, along with protein Maldi Tof MS. Among these isolates, *Bacillus* M23 and M31 were identified. In the preliminary safety screening, both strains exhibited negative hemolytic activity. Additionally, *in vitro* characteristics, such as spore formation, resistance to acid and bile salts, resistance to pathogenic microorganisms, assessment of extracellular enzyme production, and antibiotic sensitivity testing were determined for these strains, falling within the observed range for other probiotic strains. The 16S rRNA gene sequencing revealed that *Bacillus* M31 shared 97% similarity with *Bacillus clausii* DSM 8716 in the Genbank database. These findings suggest that the *Bacillus clausii* M31 shows promise as a probiotic candidate, although further extensive *in vitro/vivo* studies are necessary to validate its efficacy and safety.

Keywords: *Bacillus clausii*, probiotic, children's stools.

1 INTRODUCTION

Probiotics are defined as live microorganisms that, when administered in sufficient quantities, can impart beneficial health effects [1]. The recognition of their health benefits, such as inhibiting intestinal pathogens, promoting the growth of healthy microflora in the gastrointestinal tract, reducing cholesterol levels, controlling diarrhea, enhancing immune responses, and exhibiting anti-mutagenic and anti-carcinogenic activities, has led to a growing market for probiotic food supplements [2, 3].

While the two main genera, *Lactobacillus* and *Bifidobacteria*, are commonly represented in the market as conventional probiotics primarily isolated from sources like the gastrointestinal tract, feces, milk, and fermented foods, various species from *Streptococcus*, *Propionibacterium*, *Bacillus*, *Enterococcus*, and *Saccharomyces* from different sources are also claimed to have probiotic properties [3, 4].

Corresponding authors:
Nguyen Quynh Anh Ngo: qanhk63hup@gmail.com
Chien Ngoc Nguyen: nhibd@vui.edu.vn
Nhi Dinh Bui: chiennn@hup.edu.vn
Received: 28/02/2024 Accepted: 18/05/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2444

© 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

It is noteworthy that many accessible probiotic strains belong to lactic acid bacteria (LAB), a group of non-sporulating bacteria. However, it is crucial to recognize that spore-forming bacteria, such as *Bacillus* species, have gained significant attention due to their unique properties when compared to vegetative cells [1, 5].

Probiotic strains must meet essential standards and endure various stages of manufacturing, storage, transportation, and application [6]. Spore-forming bacteria, like *Bacillus* species, exhibit remarkable resistance to heat, UV irradiation, pH conditions, desiccation, and solvents. This resistance provides them with the capability for long-term storage at low or room temperature, increased stability in heat processing, and enhanced acid tolerance-important traits that address challenges associated with *Bacillus* usage as probiotics [4, 7, 8].

This resilience opens up the possibility of incorporating spore-forming bacteria into food products, making them potentially dominant microorganisms in pasteurized milk-based products [8]. While numerous *Bacillus* strains with probiotic potential have been studied in various *in vitro* and *in vivo* experiments, some, including *B. subtilis*, *B. polyfermenticus*, *B. clausii*, *B. coagulans*, *B. licheniformis*, and *B. pumillus*, have received approval for commercial use as dietary supplements or growth promoters in aquaculture and animals. Extensive research has focused on isolating *Bacillus* strains from diverse sources for the development to develop products [9, 10]. *Bacillus* species, especially *B. subtilis*, are prevalent in soil but have been identified in water, air, human and animal gut, vegetables, fermented foods, raw and pasteurized milk, and dairy products [3]. Consequently, their ubiquitous presence in different environments allows them to easily find their way into food products, often being part of milk microflora [4].

Recent research highlights the extensive use of these bacteria for generating metabolites, including amino acids, antibiotics, bacteriocins, surfactants, and bioactive peptides. Moreover, certain *Bacillus* species, such as *B. amyloliquefaciens*, *B. licheniformis*, *B. subtilis*, and *B. pumilus*, have gained attention for their potential as probiotics or feed additives, as evidenced by increasing proposals for their utilization in these roles [11, 12].

Bacillus clausii has been available in the market for more than 55 years and is distinguished by the presence of four probiotic strains [10]. Its resilience to various physicochemical conditions, including heat, antibiotics, and gastric pH, is attributed to the presence of spores [12, 13]. The notable resistance of *B. clausii* to a broad spectrum of antibiotics ensures that its effectiveness remains unaffected even when administered alongside antibiotic therapy [13]. Additionally, *B. clausii* stands out due to its rapid growth in both aerobic and anaerobic environments [10]. *B. clausii* spores can thrive in young shoots, undergo growth and proliferation, and subsequently regenerate spores [2, 3]. These spores can be present in various environments, including soil, straw, mud, and are also identified in the stems of insects, animals, and humans [3, 4]. Numerous studies indicate that *Bacilli* can be readily obtained from the human gastrointestinal tract through biopsy and the isolation of stool samples [3, 4]. This study focuses on isolating potential *Bacillus clausii* strains from the human digestive tract - finding a safe strain that has good acid and antibiotic resistance and can be used as a probiotic. This study focuses on isolating potential *Bacillus clausii* strains from the human digestive tract - finding a safe strain that has good acid and antibiotic resistance and can be used as a probiotic. Hence, the objective of this study was to explore the probiotic properties of the *B. clausii* M31 strain and assess its safety, with the aim of to utilize it as a raw material for the production of probiotic products.

2 MATERIALS AND METHODS

2.1 Materials

Bacillus clausii strains were isolated from stool samples of healthy children in Hai Duong Province, a northern province of Vietnam. Pathogenic bacterial strains, including *Escherichia coli* ATCC 8739,

Staphylococcus aureus ATCC 6538, *Salmonella typhimurium* ATCC 13311, and *Candida albicans* ATCC 10231, utilized in the study were provided by the National Institute of Drug Quanlity Control.

Reference Strain: *Bacillus clausii* 088AE (sourced from commercial preparations are known as SEBclausii, BioSEB CII).

All other chemicals were of reagent grade and were used without further purification such as Nutrient Broth (*NB, Himedia, India*); Luria-broth agar (*LB, Himedia, India*); Glycerol, MRS agar, MRS broth, Bile Salt; 5N hydrochloric acid (*Merck, Germany*); Tryptic Soy Agar (*TSA, Himedia, India*); Mueller-Hinton (*Himedia, India*); Sheep blood (*MELAB, Vietnam*); Brain Heart Infusion (BHI) broth (Italy).

2.2 Research Methods

2.2.1 Isolation of Probiotic Bacteria

Multiple stool samples were gathered from healthy children who had refrained from consuming probiotic products for a consecutive two-week period. Following the methodology detailed by Lee *et al*., 2019 [9], 1 g of each sample underwent homogenization in 10 mL of Nutrient Broth (NB, Himedia, India) and was subsequently heated at 80 °C for 10 min to eliminate vegetative cells. A ten-fold serial dilution of the supernatant was spread-plated onto Luria-broth agar (LB, Himedia, India), and the LB plates were incubated at 37 °C for 24 h. Individual colonies were streaked across the plate to obtain pure isolated colonies. Subsequently, bacterial isolates underwent observation for colony shape, cell morphology, and Gram stain. Distinct strains were stored at 4 °C for future use to examine probiotic properties, while the isolated Gram-positive strains were preserved in 20 % glycerol at 4 °C.

2.2.2 Biochemical identification of *Bacillus* strains using protein *MALDI TOF MS*

The isolates were tested in duplicate. Following the methods outlined by Starostin *et al.*, 2015 [14], a colony was directly spotted on the MALDI plate with 1 µL of 70 % formic acid added to each spread sample and allowed to dry. Subsequently, 1 µL of the HCCA Matrix solution (composed of 47.5 % water, 50 % acetonitrile, and 2.5 % trifluoroacetic acid) was applied to each position containing a sample. The plate was then inserted into the MALDI Biotyper and the Flex Control software (*Bruker, Germany*) was initiated. The mass spectra were acquired within 10 min. The spectra were imported into the integrated Bruker Bacterial Test Standard (*BTS, Bruker Daltonik GmbH, Bremen, Germany*). The obtained mass spectra were subsequently compared with those of known bacterial strains from commercial libraries provided by Bruker, which presently encompasses around 9000 reference bacterial proteins [15].

2.2.3 Evaluating the Ability to Form Spores

In adverse environmental conditions, *Bacillus* strains demonstrate the capability to undergo spore formation [3]. The bacterial strains were cultivated in liquid NB at a temperature of 37 ℃, with a shaking speed set at 120 rpm, over a duration for 24 h. Subsequently, the bacterial cultures underwent heat treatment at varying temperatures for a duration of 15 min, specifically at 40 ℃, 50 ℃, 60 ℃, 70 ℃, and 80 ℃. Following this treatment, the samples were appropriately diluted, and the bacterial suspension was evenly spread onto agar plates containing LB agar. The subsequent step involved the observation and quantification of the colonies proliferating on the agar plates, allowing for the calculation of bacterial density.

2.2.4 Assessment of Bile Salt Tolerance

To evaluate bile salt tolerance, *Bacillus* cultures from overnight incubation were re-suspended in sterile Phosphate-Buffered Saline (PBS) pH 7.2 (*Merck, Germany*). The suspension was then adjusted to reach a concentration of 10^8 CFU/mL. This adjusted culture was introduced into fresh MRS broth containing 0.3 % (w/v) Bile Salt and incubated for 6 h. Cell viability was determined at 0, 3, and 6 h of incubation through serial dilution and plating onto MRS agar (Jose *et al.*, 2015) [17].

2.2.5 Test for Survivability in Acid Tolerance

For the determination of acid tolerance, various pH

levels were established using a 5N hydrochloric acid solution, namely pH 1.0, 2.0, 3.0, and 4.0, in PBS. The isolates underwent incubation in NB for 18 h at 37 °C. Subsequently, the cell pellet was collected, washed in PBS, and resuspended in solutions with pH values of 2 and 4. The incubation period lasted 4 h at 37 °C. To assess survivability, plate counts on nutrient agar were conducted at 0, 1, 2, and 4 h [18]. The survival rate was determined using the following equation:

Survival rate (%)= ($N0$/$N1$)×100

$N1$ and $N0$ represent the logarithmic colony-forming units per milliliter (log cfu/mL) count of the selected species after and before treatment.

2.2.6 Assessment of Resistance to Pathogenic Microorganisms

The inhibitory effect of *Bacillus* isolates against certain pathogens was initially determined using the agar well diffusion technique [5]. *Bacillus* isolates were cultured in MRS broth at 37 °C overnight, while the target pathogens were pre-cultured under the same conditions in Brain Heart Infusion (BHI) broth (*Panreac, Italy*). Exactly 200 µL of the test pathogens (10^7 CFU/mL) were then spread onto the surface of Mueller Hinton Agar plates (*Himedia, India*).

Wells were punctured into the inoculated plates and filled with 100 µL of cell-free supernatant obtained by centrifugation of *Bacillus* cultures at 6000 rpm for 10 min (*Hettich Eba, Germany*). The plates were incubated at 37 °C for 24 h, and the antagonistic activity of *Bacillus* was assessed by measuring the formation of inhibition zones (mm) around the wells. This technique was performed in triplicate for each *Bacillus* isolate, and the mean result was recorded. Refining the methodology outlined by Amoah K *et al*., 2019 [19], the determination of resistance to deleterious bacterial and fungal strains involves the agar perforation method using the following test microorganisms: *S. aureus* ATCC 6538, *E. coli* ATCC 8739, *S. typhimurium* ATCC 13311, and *C. albicans* ATCC 10231. The observation of the agar plate was conducted after 24 - 48 h, and any inhibition rings around the agar hole were measured for diameter (D - d, mm). In this context, D represents the diameter of the inhibition ring in millimeters, and d signifies the diameter of the agar hole in millimeters [6, 18].

2.2.7 Assessing the Ability to Produce Extracellular Enzymes

The *Bacillus* strains under scrutiny were subjected to assessments for extracellular amylase, protease, and cellulase activities on plate starch-agar medium (LB give more 1% starch, *Everest-India)*, casein-agar (LB give more 1% casein, *GNC-India*), and carboxymethylcellulose (CMC)-agar media plates (LB give more 1% casein, *Wealthy - China*) , respectively. These evaluations followed the methodologies outlined by Amoozegar *et al*., 2003, and Niranjana *et al*., 2020 [21, 22]. In the screening process, each *Bacillus* strain's colony was positioned at the center of the corresponding selective medium agar plate. After the incubation period, a thorough assessment of all plates took place, with measurements taken for the diameters of the clearance zones, excluding the bacterial colony's diameter. The relative enzyme activity (REA) was calculated using the formula: REA = (D/ d) following the methodology specified by Latorre *et al*., 2016 [23]. Based on the outcomes of the REA test, organisms were classified as excellent (REA > 5.0), good (REA > 2.0 – 5.0), or weak (REA < 2.0).

D: the diameter of the zone of clearance

d: the diameter of the bacterial colony in millimeters.

2.2.8 Antibiotic Sensitivity Test

The antibiotic susceptibility of the selected *Bacillus* strains was assessed using the Kirby-Bauer disk diffusion technique, which adheres to the Clinical and Laboratory guidelines Institute 2021 (CLSI) performance guidelines for antimicrobial susceptibility testing. Several antibiotics (*Oxoid, United Kingdom*) were tested, including ampicillin (10 µg), streptomycin (10 µg), erythromycin (15 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), trimethoprim

(1.25 µg)/sulfamethoxazole (23.75 µg). Bacterial strains were cultured on Tryptic Soy Agar (Himedia, India) plates for 24 h at 37 °C. Following this, the colonies of each strain were suspended in a 0.9 % NaCl solution to prepare a concentration of approximately 1×10^8 CFU/mL. This suspension was then applied to Mueller-Hinton agar (Himedia, India) agar plates using a swab. Antibiotic discs were positioned on the seeded plates, and the zones of growth inhibition were measured after 18 h of incubation at 37 °C following methods by Spears *et al*., 2021 [24].

2.2.9 Hemolytic activity

The hemolytic activity of *Bacillus* strains was determined by using Columbia agar containing 5 % (w/v) sheep blood (*MELAB, Vietnam*). After 24 h of incubation at 37 °C, the hemolytic activity of strains was evaluated and classified based on the lysis of red blood cells in the medium around the colonies. The formation of clean and green areas on the plates was judged as hemolysis. No surrounding area should be identified as non-hemolytic activity by Thi *et al*., 2022 [25]. Alpha hemolysis (α), beta hemolysis (β), and gamma-hemolysis (γ) appeared as green-hued, clear, and no clear zones around the colonies, respectively. Strains with γ-hemolysis are considered safe.

2.2.10 Molecular identification by 16S rRNA sequencing

Separate colonies of selected *Bacillus* strains were used for DNA extraction using the DNA Blood and Tissue DNA Extraction Kit according to the manufacturer's instructions (Qiagen). The purified DNA is used as a template for PCR to amplify the gene segment encoding 16S rRNA with specific primer pairs. The DNA sequences were then analyzed using Illumina MiSeq software (*Illumina, Inc., USA*) and compared with data available in NCBI's gene bank (GenBank), using the BLAST program to find the strain with the closest sequence with a similar ratio copper. The amplification of the 16S ribosomal RNA (rRNA) gene was carried out utilizing the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525R (5'-AAAGGAGGTGATCCAGCC-3'). PCR was employed to amplify the DNA products, and the process followed these conditions: an initial denaturation at 95°C for 3 min, succeeded by 35 cycles, each comprising 95°C for 30 s, 55°C for 30 s, and 72°C for 2 min. The amplification concluded with a final extension step at 72°C for 5 min [26].

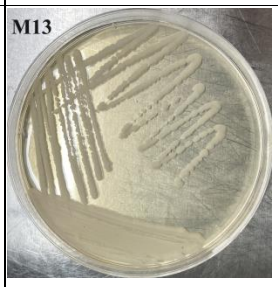
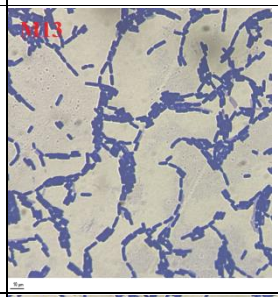
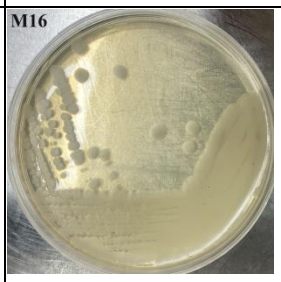
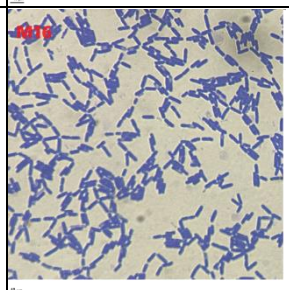
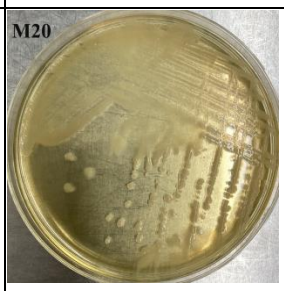
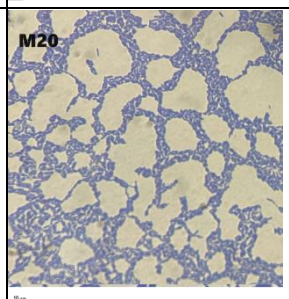
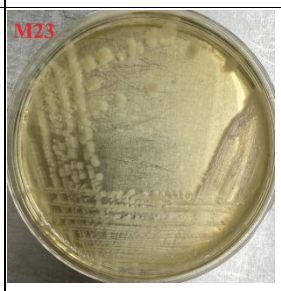
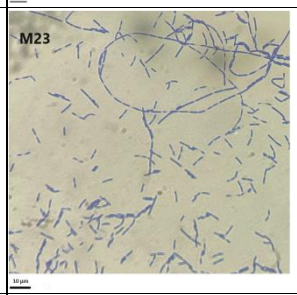
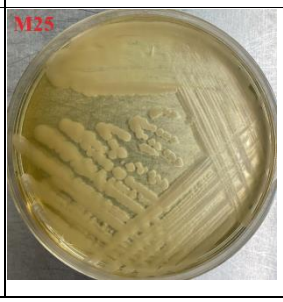
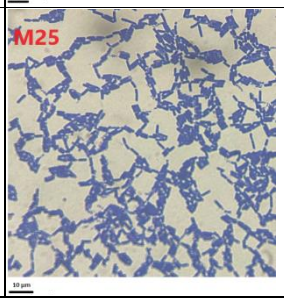
Morphological and biochemical tests such as Gram-positive, catalase and oxidase-positive, motility, API CH50 and cell cell-shaped tests were implemented following the mentioned methods before [9, 11]

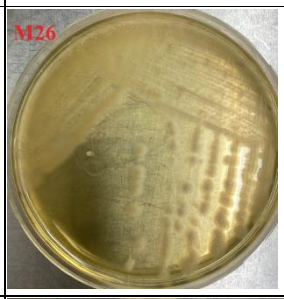
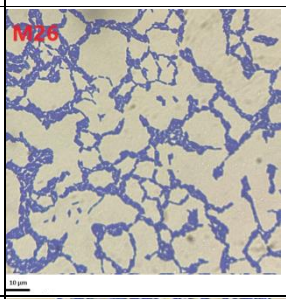
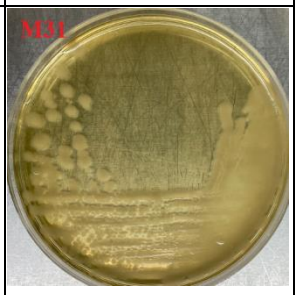
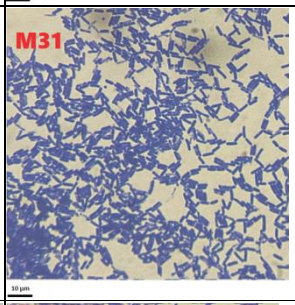
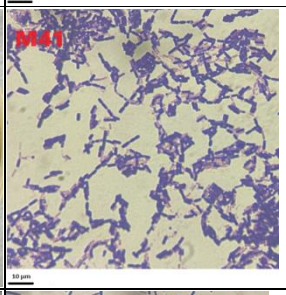
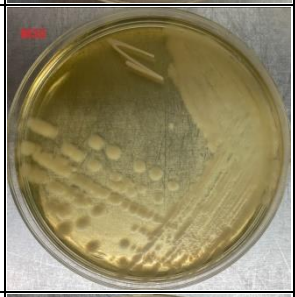
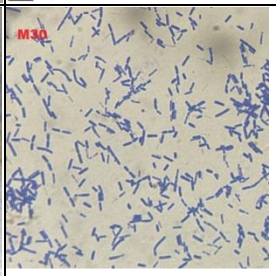
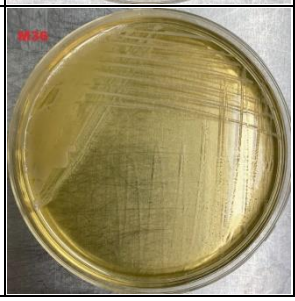
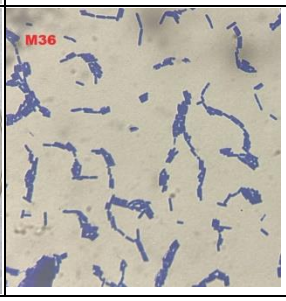
3 RESULTS AND DISCUSSION

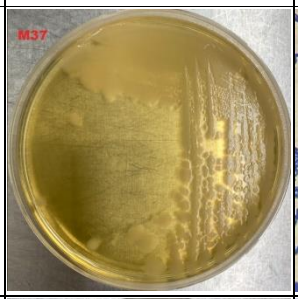
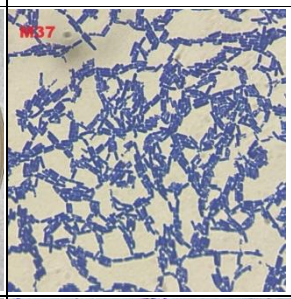
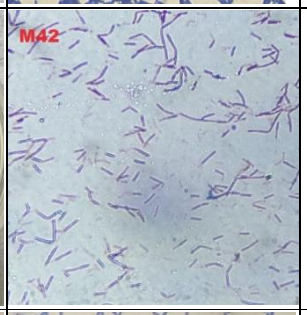
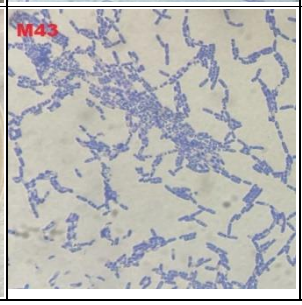
3.1 Isolation of *Bacillus*

The guidelines for identifying and choosing the most potential *Bacillus* probiotic strains for probiotic use are illustrated in *Figure 1* below. A total of 13 strains were extracted from fecal samples of healthy children, employing specific criteria such as their characteristic morphological features (small pinpointed and creamy white colonies). These strains were identified by performing morphological and biochemical tests such as Gram, catalase and oxidase-positive, motility, and coccus and rod-shaped tests [4, 12]. Initially, 13 different characteristic colonies were isolated from feces's children samples and predominantly identified using morphological and biochemical assays (*Fig. 1, Table 1*)

Table 1. Characteristics of isolated *Bacillus* strains

Strain code	(a)	(b)	(c)	(d)	(e)	(g)	*Colony image*	*Gram staining image*	API CH50
M13	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus cereus***
M16	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus cereus***
M20	(+)	(+)	(+)	(+)	(+)	(+)			***Bacillus pumilus***
M23	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus clausii***
M25	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus cereus***

Strain code	(a)	(b)	(c)	(d)	(e)	(g)	*Colony image*	*Gram staining image*	API CH50
M26	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus subtilis***
M31	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus clausii***
M41	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus amyloliquefaciens***
M30	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus cereus***
M36	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus subtilis***

Strain code	(a)	(b)	(c)	(d)	(e)	(g)	*Colony image*	*Gram staining image*	API CH50
M37	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus flexus***
M42	(+)	(+)	(+)	(+)	(+)	γ			***Bacillus licheniformis***
M43	(+)	(+)	(+)	(+)	(+)	(+)			***Bacillus pumilus***

** (a) Gram staining; (b) Catalase; (c) Motility; (d) Glucose; (e) VP test; (f) Hemolytic activity*

Results of Gram staining and microscopic observation show that the bacteria were in the form of short rods, with round ends, standing alone, sometimes joined together to form long filaments (*Fig. 1*). These bacterial strains are all Gram Gram-positive. Through the morphological characteristics of colonies, cells, the results of physiological and chemical characteristics of the strains and the API CH50 test, we can preliminarily conclude that these strains have many characteristics that overlap with *Bacillus* according to Bergey's Classification of Bacteria and Elshaghabee *et al*, 2017 [7]. *Bacillus spp.* has been considered one of the most prominent probiotics due to its better properties compared to other probiotics. The isolation of *Bacillus* bacteria in human feces samples in this study also confirmed results from previous studies suggesting that probiotics that can naturally be isolated from the human gut are likely to have the ability to survive passage through the gut [30]. *Bacillus clausii* and *Bacillus licheniformis* have been isolated from healthy human adult feces, indicating their ability to survive passage through the gastrointestinal tract [31].

3.2 Results of *Bacillus* identification using MALDI TOF MS protein mass spectrometry technology

Mass spectra obtained from each tested species were compared to each other. For each species, a master spectrum (MSP) was generated and included in the reference library. In the program created by MSP using

Flex software from Bruker Daltonik, Bremen, Germany, the *Bacillus* strain protein mass spectra are placed on a separate branch from the branch containing most of the protein mass spectra of *Bacillus spp.* species, and the results are returned. The obtained identification results using the MALDI TOF protein mass spectrometry method in *Table 2* show that the strains identified are *Bacillus* spp. Among them, M23 and M31, identified as *Bacillus clausii*, are the strains being sought. The remaining strains are identified as *Bacillus subtilis*, *Bacillus cereus* etc. The experiments were repeated three times (n=3) for a high confidence level (confidence level, also known as the threshold criterion, set at 2.0 by Bruker for safe genus identification, determined to the species level). These identification results are likely to be reliable and consistent with previous research results of authors such as Starostin *et al.*, 2015 and Manzulli *et al.*, 2021, which has have demonstrated a high degree of reproducibility [14, 32].

Table 2. Results of strain identification test by MALDI TOF MS

Strain	**Detected species**	***Score****
M13	*Bacillus cereus*	2.23
M16	*Bacillus cereus*	2.15
M20	*Bacillus pumilus*	2.13
M23	*Bacillus clausii*	2.26
M25	*Bacillus cereus*	2.23
M26	*Bacillus subtilis*	2.15
M30	*Bacillus cereus*	2.13
M31	*Bacillus clausii*	2.25
M36	*Bacillus subtilis*	2.02
M37	*Bacillus flexus*	2.01
M41	*Bacillus amyloliquefaciens*	2.11
M42	*Bacillus licheniformis*	2.13
M43	*Bacillus pumilus*	2.04

** The experimental results were repeated n=3; high level of confidence (confidence level, also known as threshold criterion > 2.0, defined by Bruker as "safe genus identification, determined to the species level")*

The characteristic spectrum called PMF (Peptide Mass Signature) is generated for the analytes in the sample. Bacterial identification is performed by comparing the PMF of the unknown organism with the PMF present in the database or by comparing multiple biomarkers of the unknown organism with the proteome database. The MALDI-TOF method is often used for simple protein samples, and is quite pure. The advantage is that it can sequence amino acids and peptide fragments and accurately measure mass, but it is not suitable for analyzing complex protein mixtures. DNA has been extracted to be pure and from there, analyzed to the species level with highly reliable score results (using the MALDI TOF protein mass spectrometry method). This is also the basis for performing further experiments.

3.3 Evaluating the ability to form spores

After time from cell stimulation at increasing temperatures, the bacterial strains have an increased ability to produce spores and have relatively high spore density [30]. At 80°C after 15 min, strains M23 and M31 have high spore formation ability.

Table 3. Results of evaluatingAssessing the ability of bacterial strains to produce spores. the spore production ability of bacterial strains

Strain code	Rate of spore formation (log CFU/mL)				
	40°C	**50°C**	**60°C**	**70°C**	**80°C**
Bacillus M23	0.0075 ± 0.001	1.35 ± 0.21	5.19 ± 0.23	7.40 ± 0.22	9.64 ± 0.28
Bacillus M31	0.0264 ± 0.001	1.62 ± 0.17	5.21 ± 0.12	7.64 ± 0.16	10.54 ± 0.36
Bacillus clausii 088AE	0.0318 ± 0.001	1.71 ± 0.34	5.15 ± 0.24	7.31 ± 0.23	10.04 ± 0.16

Note: Cultures started with spores (10^8 *CFU/mL), in nutrient broth, at 37°*C.

Mean values with SE from three biophotometer experiments

A number of other studies also confirmed that *Bacillus spp*. spores are formed, but the specific quantity is not mentioned [30, 31]. In this study, we found that the number of spores of *Bacillus* M23, M31 was relatively high after 15 min of heating and gradually increased as the temperature increased. The ability to sporulate is a crucial characteristic of *B. clausii* to sustain effects on the digestive tract, as vegetative cells are lost when the pH drops below 2. In this study, the spore production rate of M23 and M31 was approximately 10^{10} CFU/mL when the medium was stimulated at high temperatures. However, the spore formation rate of M23 is weaker than that of M31. M31 is nearly equivalent to the reference *B. clausii* 088AE. And M31 at 80°C reached the highest number of about 10^{10} CFU/mL, with high potential in probiotic products.

3.4 Survivability in Simulated in Bile Salts

Bacillus M23 and M31 were tested for their ability to grow in 0.3% bile salts for varying times, an important taxonomic characteristic of the species. The bile salt tolerance of all tested bacteria was confirmed and there were minor differences between strains.

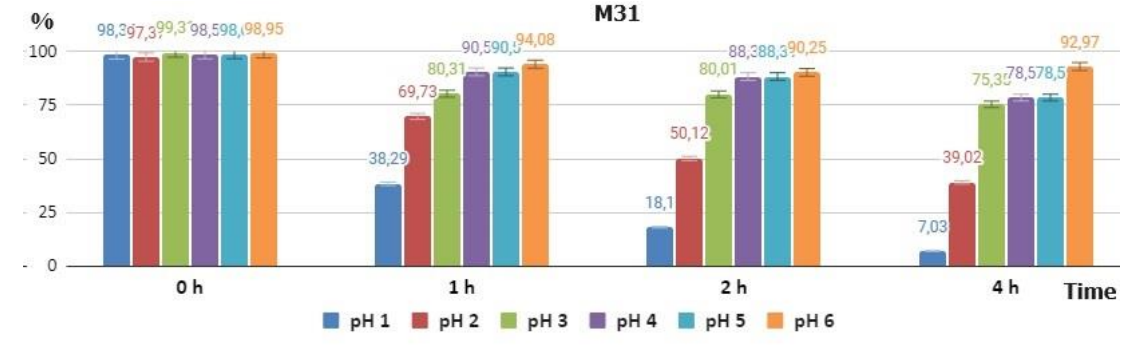
Table 4. Viability of the *Bacillus* isolated strains (log10 CFU/mL) after 0, 3 h, and 6 h of incubation in 0.3% bile salt

Strain code	Bile salt		
	0 h	**3 h**	**6 h**
Bacillus M23	8.96 ± 0.06	8.97 ± 0.03	9.32 ± 0.09
Bacillus M31	8.92 ± 0.05	8.93 ± 0.05	9.65 ± 0.05
B. clausii 088AE	8.95 ± 0.05	8.96 ± 0.05	9.75 ± 0.05

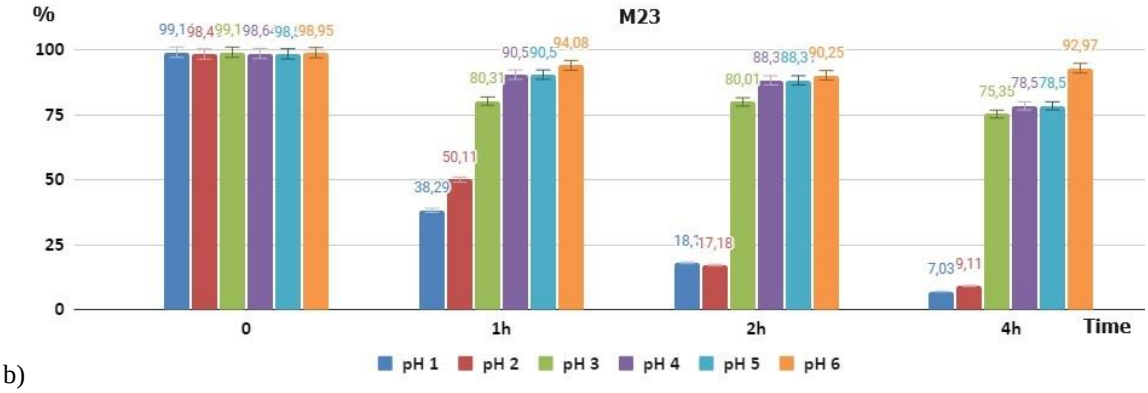
The survivability and growth of potential probiotic strains in the gastrointestinal tract (GIT) depend significantly on their capability to endure and resist intestinal bile salts. Therefore, the ability to tolerate bile salts is a crucial criterion for selecting probiotics [15]. Many studies have assessed the bile salt tolerance of potential probiotics, commonly using an average level of 0.3 % bile salt in their evaluations [16]. In this study, two *Bacillus* isolates, namely M23 and M31, exhibited robust tolerance to 0.3 % bile salt after a 6 h exposure. There was no significant difference ($P > 0.05$) in the viability of *Bacillus* strains between 3 h and 6 h of incubation. Additionally, the range of viability for *Bacillus* strains in the bile salts environment, measured in log10 CFU/mL, after a 6 h incubation, varied from 9.32 ± 0.09 to 9.65 ± 0.05. This was comparable to the recorded range of 8.95 ± 0.05 to 9.75 ± 0.05 (*Table 4*).

3.5 Results of acid tolerance survey

Probiotic bacterial strains exhibit a critical attribute of resilience to low acidity, a prevalent condition in the upper gastrointestinal tract [12, 15]. Consequently, from the isolated bacterial strains, their capacity to endure varied pH conditions (ranging from pH 1 to 7) in nutritional environments was investigated at different time points, specifically 0 h, 1 h, 2 h and 4 h. The findings indicate that the two identified *Bacillus* strains demonstrated noteworthy tolerance to low pH levels (pH 1, 2, 3, 4, 5, and pH 6), along with 0.3 % bile salts, as assessed. Both strains exhibited survival under acidic conditions, signifying a high level of tolerance in these isolates. Additionally, both strains displayed resistance to bile salts, as detailed in *Fig 3*.



a)



b)

Fig 2. Survival of *Bacillus* strains M23 and M31 in culture environments with different pH levels

a) Survival of Bacillus M31 in different pH conditions

b) Survival of Bacillus M23 in different pH conditions

Note: Cultures started with spores (10^8 *CFU/mL), in nutrient broth different pH, at 37*°C.

Mean values with SE from three biophotometer experiments

At pH 1, both strains decreased in number rapidly, proving that *Bacillus* cannot tolerate pH 1 environment. After only 1 hour, the number decreased by less than 40%. At pH 2, *Bacillus* M31 after 1 h survival 70%, after 4 h remains 39%. However, *Bacillus* M23 survival sharply to 40% after just 1 h, proving that the digestive tract's survival ability to withstand harsh conditions is not good. Next, at pH 3, two strains M23 and M31 both survived about 80% after 4 h. Similar to pH 4 and pH 5, survival is more than 80% after 4 h. With pH 6, the survival rate is higher than

about 90%. Among the two strains of *Bacillus clausii*, which have undergone preliminary identification via biochemistry and MALDI TOF, it has been observed that they exhibit growth capability at levels below pH 3. Notably, M31 demonstrates resilience and pronounced resistance at acidic pH levels. Prior investigations conducted by Sorokulova *et al*. (2013) and Amoah *et al*. (2021) have elucidated that probiotic bacterial strains within the *Bacillus* genus can endure acidic conditions ranging from pH 2 to pH 3 for a duration of 2 h [4, 19]. Moreover, Piggo *et al*. (2008) documented the capacity of *Bacillus* genus bacterial strains to endure pH 3 for 120 min [33]. In our study, bacterial strains M23 and M31 exhibit the ability to withstand pH 2 for an equivalent duration of 2 h. Notably, M31 demonstrates a heightened advantage, displaying the highest bacterial survival density when subjected to acidic pH conditions and survival in pH 1/1h around 30%; in pH 2/ 2h around 70% was better survival ability than others studies. This result also shows that it is necessary to protect isolated *Bacillus* strains at lower pH to achieve better effects on the digestive tract.

3.6 Evaluating antibacterial ability

Bacillus strains M23 and M31 were assessed for their antibacterial activity using the agar well diffusion method. The capability to resist pathogenic microorganisms is a significant classification characteristic of probiotics. The antibacterial effectiveness of all tested bacteria was verified. There were minor distinctions between the strains, as illustrated in *Table 5*.

Table 5. Antibacterial activity of *Bacillus* M23, M31 strains using well diffusion method

Strain code	Inhibition zone (mm)		
	Bacillus **M23**	*Bacillus* **M31**	*B. clausii* 088AE
***S. aureus* ATCC 6538**	8.26 ± 0.21	13.50 ± 0.41	12.43 ± 0.24
***E. coli* ATCC 8739**	10.17 ± 0.25	12.58 ± 0.40	12.38 ± 0.23
***S. typhimurium* ATCC 3311**	9.33 ± 0.61	9.52 ± 0.81	10.70 ± 0.60
***C. albicans* ATCC 10231**	11.24 ± 0.13	12.93 ± 0.47	12.67 ± 0.22

The assessment of antibacterial efficacy for the isolated *Bacillus* M23 and M31 strains involved the measurement of the growth inhibition zone diameter against various pathogenic bacteria within an agar plate environment supplemented with probiotics. The findings presented in *Table 5* indicate that *Bacillus* M31 exhibits superior effectiveness in restraining the proliferation of both Gram-positive and Gram-negative pathogenic bacteria with *Bacillus* M31 and *B. clausii* 088AE. Additionally, it demonstrates notable inhibitory capabilities against fungal growth [7, 15, 35]. Inhibition zones of *Bacillus* M31 were larger than *Bacillus* M23 and similarly *B. clausii* 088AE with *E. coli* ATCC 8739; and larger than *B. clausii* 088AE with *S. aureus* ATCC 6538. This result is similar to the results of evaluating Bacillus strains isolated from other sources such as poultry feces, pig feces, fish or other human gastrointestinal tract [5, 8, 19, 21]. Furthermore, in this study, *Bacillus* strains M23 and M31 showed larger antibiotic inhibition zone larger. Furthermore, in this study, *Bacillus* strains M23 and M31 showed antibacterial inhibition zone larger than related isolated *Bacillus* from other sources (12.93mm with *C. albicans* ATCC 10231)

3.7 Assessing the ability to produce extracellular enzymes

The enzyme production ability was assessed by calculating the ratio of the hydrolysis circle diameter (D) to the colony diameter (d). Investigation of the ability to produce extracellular enzymes using the agar well method was performed with bacterial *Bacillus* M23 and M31.

Table 6. Relative enzyme activity values produced by selected *Bacillus* M23 and M31 *Relative enzyme activity (REA)*

Strain	Ring diameter (mm)	
	Amylase	***Protease***
Bacillus **M23**	1.50 ± 0.21	3.00 ± 0.14
Bacillus **M31**	1.57 ± 0.13	2.60 ± 0.15
B. clausii 088AE	2.00 ± 0.11	2.75 ± 0.17

Bacillus M23 and M31 are both capable of producing amylase and protease enzymes. The amylase production ability of the strains is lower than the protease production ability, showing that the diameter of the starch substrate degrading circle (1.5 - 2.0 mm) is higher than the casein degrading ring diameter (2.6 - 3.0 mm). This result is also consistent with some previous studies, therefore *B. clausii* strain has the ability to produce extracellular enzymes to degrade substrates [21, 34]**.**

3.9 Antibiotic sensitivity testing

Bacillus M23 and M31 were tested for antibiotic sensitivity. Determining whether probiotics are antibiotic resistant or not is an important classification characteristic. The information of *Table 7* illustrated the antibiotic resistance of all tested bacteria was recorded and the differences between M23 and M31.

Table 7. Antibiotic sensitivity profiles of *Bacillus* strains

Antibacteria	Strain	
	Bacillus **M23**	*Bacillus* **M31**
Ampicillin 10 µg	26.08±1.10* (S)**	27.16±1.45 (S)
Streptomycin 10 µg	18.02±0.71 (S)	30.27±1.61 (S)
Erythromycin 15 µg	21.02±0.60 (S)	24.17±1.18 (S)
Tetracycline 30 µg	26.10±1.08 (S)	34.00±1.20 (S)
Chloramphenicol 30 µg	23.05±1.41 (S)	26.03±1.11 (S)
Ciprofloxacin 5 µg	20.27±1.14 (S)	25.67±1.12 (S)
Gentamycin 10 µg	26.15±1.20 (S)	28.01±1.09 (S)
Trimethoprim 1.25 µg/ Sulfamethoxazole 23.75 µg	17.36±0.80 (S)	20.24±1.21 (S)

** Mean±SD expressing data of inhibition diameter (mm) in three replications.*

*** Zone Diameter Breakpoints, nearest whole (mm): Sensitive (S), Resistance (R)*

The antibiotics utilized in this investigation were evaluated in studies by Guo *et al*., 2016 [38]. The antibiotic resistance and sensitivity levels of isolated *Bacillus* strains were assessed using the CLSI 2020 standard values and the specific S and R indices were different for each type of antibiotic. *Table 7* results are also consistent with earlier research showing that *Bacillus* M23 and M31 isolates are particularly sensitive (S) to numerous antibiotics [40]. Furthermore, the recovered *Bacillus* strain was sensitive to Ciprofloxacin, Chloramphenicol, Streptomycin, and Tetracycline, which was consistent with the findings of other studies such as Abbrescia *et al*., 2018, Guo et al., 2016, Cutting M., 2020 [38, 39, 40].

3.8 Evaluation of hemolytic activity

In accordance with FAO Animal Production and Health [29], it is advisable for microbial strains intended for use as probiotics to be safe within the host. Choosing and utilizing strains that lack haemolytic activity as probiotics highlights their non-virulent characteristics. The results of testing hemolytic activity with isolated strains are shown in *Table 1*. Among them, two hemolytic strains M20 and M43 were eliminated from subsequent

experiments. Out of the examined *Bacillus* strains for haemolytic activity, demonstrated non-haemolytic behavior, making them suitable for further evaluation due to their safety as probiotics. This aligns with prior findings that indicate a significant portion of bacterial strains had non-haemolytic properties [18, 28]. Evaluation of potential *Bacillus clausii* strains including *Bacillus* M23 and M31 did not show hemolytic activity, the results of culture on agar plates are shown in *Fig 4* below.

a)*Bacillus clausii* 088AE **b)*Bacillus* M31** **c)*Bacillus* M23**

Fig 4. Hemolytic activity of *Bacillus* strains M23, M31

Detection of hemolytic activity is regarded as a crucial indicator of virulence and serves as a fundamental safety assessment when pre-screening a strain for potential investigation as a probiotic or feed additive. The European Food Safety Authority (EFSA) strongly advises the assessment of hemolytic activity to ensure that a bacterial strain, even if it holds Generally Recognized As Safe (GRAS) or Qualified Presumption of Safety (QPS) status, is devoid of toxigenic potential (Yasmin *et al.*, 2020) [41]. Strains displaying hemolytic activity may be deemed unsuitable for applications in human or animal health until the impact of this virulence factor is either mitigated, altered, or confirmed as non-harmful to the eukaryotic host. In the present study, *Bacillus* M23 and M31 were examined, revealing no transparent or greenish zones around their colonies on blood agar plates. This observation contrasts with the henomenon noted in control strains of *Bacillus clausii* 088AE.

3.9 Results of gene sequencing using the 16S rRNA method

The 16S rRNA gene sequence region of the bacterial strains was amplified by PCR and sequenced. With the primer pair used, it was shown that this gene region was amplified in all bacterial strains. The size of the amplified fragment is about 350-400 bp.

The 16S rRNA gene sequence of the bacterial strains was used to build a phylogenetic tree, combining the 16S rRNA gene sequence region with reference bacterial strains in Genbank (*Fig. 5*). Analysis results from the phylogenetic tree of strain M31 are in the same branch as strain *Bacillus clausii* DSM 8716 with a bootstrap ratio of 97% in Genbank database under accession number CP019905.1, with a high level of confidence. This is also consistent with the studies of Simon *et al.*, 2011 and Huynh A *et al.*, 2009 when demonstrating that Bacillus bacterial strains are present in the human intestinal tract [5, 15, 17].

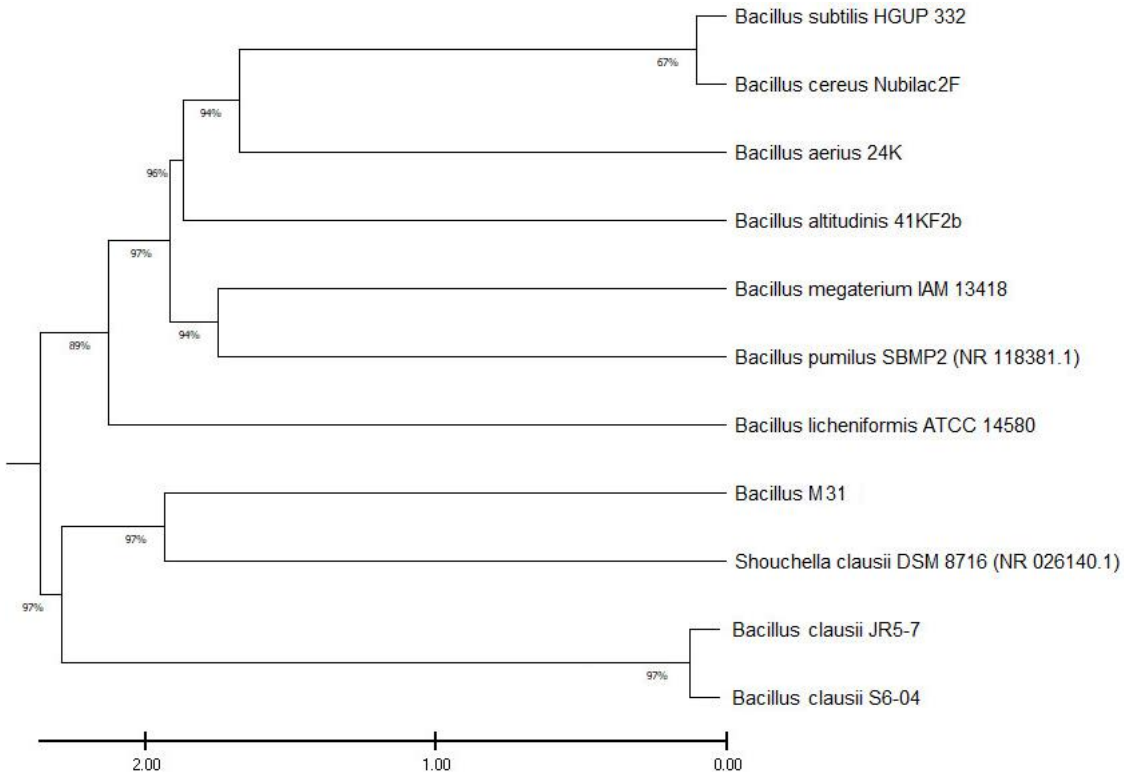


Fig 5. Phylogenetic tree of Bacillus M31 strain

4 DISCUSSION

The selective examination of *Bacillus* strains isolated from the stools of healthy children in this study highlights their distinct probiotic properties, particularly in the case of *Bacillus clausii*, showcasing their ability to thrive and flourish within the human gastrointestinal tract [1, 2]. The *Bacillus clausii* M31 strain, specifically isolated in this study, demonstrates proficiency in spore formation, resilience in challenging environments like low pH and bile salts, and resilience in the digestive tract. This strain proves beneficial in the digestion and hydrolysis of carbohydrates and amino acids, making it a viable candidate for probiotic applications in the food industry, as previously documented in other research studies [9, 20]. Thus, based on the most recent studies and available reports, there is a noticeable absence of data regarding the isolation of *Bacillus* probiotics from children's stools. Consequently, this study aimed to fill this gap by preparing children's stool samples for the isolation of *Bacillus* strains with potential probiotic properties as a novel source. The isolates, identified through biochemical and molecular testing, were associated with *B. clausii* and subsequently utilized for probiotic assessment.

It is crucial to emphasize that the probiotic attributes are inherently specific to each strain, contingent upon both the source of isolation and the intended target. Therefore, evaluating *in vitro/vivo* probiotic properties becomes a pivotal step in considering a microorganism as a probiotic, a point consistently highlighted in various studies [42–44]. Hemolytic activity, often considered detrimental to host cells and tissues [45], necessitates screening bacteria for these products to ensure the safety of an isolate [43]. Fortunately, neither of the two strains in this study exhibited hemolytic activity, aligning with similar

observations reported for probiotic candidates such as *B. clausii* UBBC07 and *Bacillus* BS 3 and BS 31 [4]. Comparable results were also reported by Keubutornye *et al*., 2017 [34], and Naeem *et al*.'s investigation on potential probiotics of *Bacillus* strains showed no hemolysis for their isolates [45].

The evaluation of antibiotic susceptibility is a crucial aspect to guarantee that bacterial strains are not resistant to antibiotics. Understanding the transfer of antibiotic resistance determinants is equally essential for investigating safe probiotics [27]. Antibiotic resistance modeling for *Bacillus* M23 and M31 demonstrated susceptibility, confirming their non-resistance to antibiotics [28]. This aligns with previous studies revealing the sensitivity of *Bacillus* strains to antibiotics [26, 29]. The antimicrobial capability is an undeniably crucial attribute of potentially probiotic bacterial strains. We demonstrated that *B. clausii* M31 has the capacity can impede the growth of opportunistic pathogens, namely *E. coli, S. aureus,* and *P. aeruginosa*, during co-cultivation in a liquid medium. Probiotic strains of *B. clausii* are recognized for their production of substances with antimicrobial properties, with some of these substances having been identified and characterized. Notably, *B. clausii* has been observed to generate antimicrobial substances effective against both Gram-negative and Gram-positive species during whey fermentation [42], underscoring the significance of the starting substrates in the synthesis of antimicrobials. Another noteworthy instance of indirect antimicrobial activity was reported by Ripert G *et al*., 2016 [46].

Given that acid and bile salts in the stomach and intestine represent the initial biological barriers that probiotic strains must overcome after ingestion, acid, and gastric juice tolerance, as well as bile resistance, are paramount factors for the viability and growth of probiotic strains during their journey through the gastrointestinal tract [44]. Both isolates in this study exhibited tolerance to acidic pH and artificial gastric juice conditions, and both strains demonstrated resistance to bile salts. These findings are in line with previous results regarding *Bacillus* strains with probiotic potential [48, 49]. The spores of *Bacillus clausii* M31 exhibited remarkable resilience against simulated gastric and small intestinal environments, suggesting their potential to endure the passage through the upper gastrointestinal tract. Earlier studies have shown that spore formulations of widely recognized *B. clausii* probiotic strains can withstand pH 2 and up to 0.3% bile salts. Furthermore, these spores can germinate and multiply in conditions mimicking the human intestinal environment [50, 51]. The sporulation rate results of M31 show good sporulation ability, which can be further improved through optimization of culture conditions, such as aeration and cell density, in industrial bioreactors. In addition, our findings confirm that the spores of *B. clausii* M31 can endure simulated gastric and small intestinal conditions, too.

Traditional methods for microbial identification, such as biochemical tests and DNA sequencing, are known for their time-consuming and labor-intensive nature. In contrast, the recently employed MALDI TOF mass spectrometry method proves to be simple and rapid, aiding in narrowing down the scope of strain evaluation to save time and costs. However, the effective application of mass spectrometry necessitates a comprehensive reference database and specific software for spectral comparison. Despite this, the identification of isolates through morphological characteristics, biochemical testing, or MALDI TOF still requires further confirmation through tests involving 16S rRNA gene sequencing [21, 23]. Classification of bacterial strains on the phylogenetic tree based on the bootstrap index corresponding to each branch. The higher the bootstrap index, the greater the confidence level that the bacterial strain to be identified is similar to the bacterial strain on the same branch. According to Hillis and Bull (1993), the confidence level of phylogenetic analysis based on the bootstrap index is conventionally defined as: bootstrap index < 65: low confidence level; 65

≤ bootstrap index < 85: average confidence level; Bootstrap index ≥ 85: high confidence level [22].

In conclusion, *B. clausii* M31 emerges as a promising candidate for probiotic applications. This designation is grounded in a comprehensive analysis of test results, which consistently demonstrated the strains' favorable probiotic potential. Nevertheless, for a definitive determination regarding their suitability as probiotic strains, further *in vitro* and *in vivo* assessments are essential. These evaluations should encompass factors such as enzymatic activity, co-aggregation, antimicrobial activity, biofilm formation, cholesterol reduction, and animal models, paving the way for a more conclusive decision on their application as probiotics.

5 CONCLUSION

Out of the thirteen isolated *Bacillus* strains examined in this study, two were identified as *Bacillus clausii*. Subsequently, the identification of *Bacillus clausii* M31 was refined based on an assessment of both the pharmaceutical product type and its corresponding activity, establishing it as the most viable candidate. Strain M31 exhibited a nucleotide sequence closely resembling *Bacillus clausii* DSM 8716, displaying 97% sequence similarity. The nucleotide sequence for *Bacillus clausii* M31 has been documented in the NCBI GenBank. *B. clausii* M31 is characterized as a Gram-positive bacillus with spore-forming capabilities. It demonstrates resilience under acidic conditions (pH 3) for a duration of 3 h, resistance to bile salt environments, resistance to pathogenic bacteria, susceptibility to antibiotics, and proficiency in amylase and protease production. Notably, *Bacillus clausii* M31 does not induce hemolysis. Finally, it can be concluded that *B. clausii* M31 could be notable as probiotic candidates.

ACKNOWLEDGMENTS

The authors would like to thank Hanoi University of Pharmacy and Haiduong Central College of Pharmacy for facilitating this study. The several writers whose work was included intoin this review are also acknowledged by the authors.

Credit authorship contribution statement

Nguyen Quynh Anh Ngo: writing of the original draft, conceptualization, methodology, writing - review & editing, supervision; Chien Ngoc Nguyen : methodology, data curation, formal analysis, writing - review & editing, and project administration; Xuan Thanh Dam: methodology, formal analysis, and project administration.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Lefevre M., et al. Safety assessment of *Bacillus subtilis* CU1 for use as a probiotic in humans. *Regul. Toxicol. Pharmacol.* 2017; 83:54–65. https://doi.org/10.1016/j.yrtph.2016.11.010
2. Kim B. J., Hong J. H., Jeong Y. S., and Jung H. K. Evaluation of two *Bacillus subtilis* strains isolated from Korean fermented food as probiotics against loperamide-induced constipation in mice. *J. Korean. Soc. Appl. Biol. Chem.* 2014; 57(6):797–806. https://doi.org/10.1007/s13765-014-4106-0
3. Hameed A., et al. Isolation and characterization of a cholesterol-lowering bacteria from *Bubalus bubalis* raw milk. *Fermentation.* 2022; 8:163. https://doi.org/10.3390/fermentation8040163.
4. Sorokulova I. Modern status and perspectives of *Bacillus* bacteria as probiotics. *J. Prob. Health.* 2013; 1:e106. https://doi.org/10.4172/2329-8901.1000e106.
5. Naeem M., et al. Screening of cattle gut-associated *Bacillus* strains for their potential use as animal probiotics. *Indian J. Anim. Res.* 2018.

https://doi.org/10.18805/ijar.B-948.

6. Barakat M., Al-Najjar M. A., Abdulrazzaq S., Talib W. H. and Athamneh T. The immunostimulatory effect of probiotic conditioned medium on RAW264.7 murine macrophages. *Jordan Journal of Pharmaceutical Sciences.* 2023; 16(2):462. https://doi.org/10.35516/jjps.v16i2.1501
7. Elshaghabee F. M. F., Rokana N., Gulhane R. D., Sharma C. and Panwar H. *Bacillus* as potential probiotics: Status, concerns, and future perspectives. *Front. Microbiol.* 2017; 8:1490. https://doi.org/10.3389/fmicb.2017.01490.
8. Permpoonpattana P., Hong H. A., Khaneja R. and Cutting S. M. Evaluation of *Bacillus subtilis* strains as probiotics and their potential as a food ingredient. *Benef. Microbes.* 2017; 3(2):127–135. https://doi.org/10.3920/BM2012.0002
9. Lee N. K., Kim W. S. and Paik H. D. *Bacillus* strains as human probiotics: Characterization, safety, microbiome, and probiotic carrier. *Food. Sci. Biotechnol.* 2019; 28:1297–1305. https://doi.org/10.1007/s10068-019-00691-9
10. Urdaci M. C., Bressollier P. and Pinchuk I. *Bacillus clausii* probiotic strains: Antimicrobial and immunomodulatory activities. *J. Clin. Gastroenterol.* 2004; 38(6):86. https://doi.org/10.1097/01.mcg.0000128925.06662.69
11. Dilshad R. and Batool R. Antibacterial and antioxidant potential of *Ziziphus jujube*, *Fagonia arabica*, *Mallotus phillipensis*, and *Hemidesmus indicus*. *Jordan Journal of Pharmaceutical Sciences.* 2022; 15(3):413–427. https://doi.org/10.35516/jjps.v15i3.417
12. Ocaña A. M. Review of *Bacillus clausii* and its use in clinical practice. *In Focus.* 2007. https://doi.org/10.1186/s40794-019-0089-5
13. Odo K. E., Agbo M. O. and Osadebe P. O. Extract and fractions from soil bacteria (*Streptomyces canus* ATCC 12647) possess antimicrobial and antioxidative potential *in vitro*. *Jordan Journal of Pharmaceutical Sciences.* 2022; 15(3):405–412. https://doi.org/10.35516/jjps.v15i3.416
14. Starostin K. V., et al. Identification of *Bacillus* strains by MALDI TOF MS using a geometric approach. *Sci. Rep.* 2015; 5:16989. doi: 10.1038/srep16989 https://doi.org/10.1038/srep16989
15. Vecchione A., Celandroni F., Mazzantini D., Senesi S., Lupetti A. and Ghelardi E. Compositional quality and potential gastrointestinal behavior of probiotic products commercialized in Italy. *Frontiers in Medicine.* 2018; 5. https://doi.org/10.3389/fmed.2018.00059
16. Suva M., Sureja V. P. and Kheni D. B. Novel insight on probiotic *Bacillus subtilis*: Mechanism of action and clinical applications. *J. Curr. Res. Sci. Med.* 2016; 2:65–72. https://www.researchgate.net/publication/312317617
17. Jose N. M., Bunt C. R. and Hussain A. M. Comparison of microbiological and probiotic characteristics of lactobacilli isolates from dairy food products and animal rumen contents. *Microorganisms.* 2015; 3:198–212. https://doi.org/10.3390/microorganisms3020198
18. Corcoran B. M., Stanton C., Fitzgerald G. F., and Ross R. P. Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars. *Appl Environ Microbiol*. 2005; 71(6):3060–3067. https://doi.org/10.1128/AEM.71.6.3060-3067.2005
19. Amoah K., Huang Q. C., Tan B. P., Zhang S., Chi S. Y. and Yang Q. H. Dietary supplementation of probiotic *Bacillus coagulans* ATCC 7050 improves growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp (*Litopenaeus vannamei*). *Fish Shellfish Immunol.* 2019; 87:796–808. https://doi.org/10.1016/j.fsi.2019.02.029
20. Giua C., Romano F., Keber E., et al. A prospective real-world study of *Bacillus clausii* evaluating use, treatment habits, and patient satisfaction in Italian community pharmacies: The PEGASO Study. *Drugs - Real World Outcomes.* 2023. https://doi.org/10.1007/s40801-023-00402-1

21. Amoozegar M., Malekzadeh F. and Malik K. A. Production of amylase by a newly isolated moderate halophile, *Halobacillus* sp. strain MA-2. *J. Microbiol. Methods.* 2003; 52:353–359. https://doi.org/10.1016/S0167-7012(02)00191-4
22. Niranjana J. and Bavithra P. S. I. A comparative study on screening methods for the detection of protease activity-containing bacteria. *Int. J. Sci. Res.* 2020; 9:169–171. https://doi.org/10.21275/ART20203705
23. Latorre J. D., Hernandez-Velasco X., Wolfenden R. E., Vicente J. L., Wolfenden A. D., Menconi A., Bielke L. R., Hargis B. M. and Tellez G. Evaluation and selection of *Bacillus* species based on enzyme production, antimicrobial activity, and biofilm synthesis as direct-fed microbial candidates for poultry. *Front. Vet. Sci.* 2016; 3:95. https://doi.org/10.3389/fvets.2016.00095
24. Spears J. L., Kramer R., Nikiforov A. I., Rihner M. O. and Lambert E. A. Safety assessment of *Bacillus subtilis* MB40 for use in foods and dietary supplements. *Nutrients.* 2021; 13:733. https://doi.org/10.3390/nu13030733
25. Thi Lan Anh H., Thi Thanh Hue L., Hai Linh B. N., Tuan Dung N. H., Duong Minh D., Thi Le Quyen T. and Trung T. T. *In vitro* safety evaluation of *Bacillus subtilis* species complex isolated from Vietnam and their additional beneficial properties. *Vietnam Journal of Biotechnology.* 2022; 20(4):727–740. https://doi.org/10.15625/1811-4989/16917
26. Mohkam M., et al. Multifaceted toxin profile of *Bacillus* probiotic in newly isolated *Bacillus* spp. from soil rhizosphere. *Biologia.* 2020; 75:309–315. https://doi.org/10.2478/s11756-019-00357-1
27. Hoyles L., Honda H., Logan N. A., Halket G., La Ragione R. M., and McCartney A. L. Recognition of greater diversity of *Bacillus* species and related bacteria in human faeces. *Res. Microbiol.* 2012; 163:3–13. https://doi.org/10.1016/j.resmic.2011.10.004
28. Lopetuso L. R., Scaldaferri F., Franceschi F., and Gasbarrini A. *Bacillus clausii* and gut homeostasis: State of the art and future perspectives. *Expert Rev. Gastroenterol. Hepatol.* 2016; 10:943–948. https://doi.org/10.1080/17474124.2016.1200465
29. Bajagai Y. S., Klieve A. V., Dart P. J. and Bryden W. L. (Eds). *Probiotics in Animal Nutrition – Production, Impact and Regulation.* Rome: FAO Animal Production and Health; 2016. 179.
30. Fakhry S., Sorrentini I., Ricca E., De Felice M. and Baccigalupi L. Characterization of spore-forming bacilli isolated from the human gastrointestinal tract. *Journal of Applied Microbiology*, 2008; 105(6):2178–2186. https://doi.org/10.1111/j.1365-2672.2008.03934.x
31. Cenci G., Trotta F., and Caldini G. Tolerance to challenges mimicking gastrointestinal transit by spores and vegetative cells of Bacillus clausii. *Journal of Applied Microbiology*, 2006; 101(6):1208–1215. https://doi.org/10.1111/j.1365-2672.2006.03042.x
32. Manzulli V., Rondinone V., Buchicchio A., Serrecchia L., Cipolletta D., Fasanella A., Parisi A., Difato L., Iatarola M., et al. Discrimination of Bacillus cereus group members by MALDI-TOF mass spectrometry. *Microorganisms.* 2021; 9(6):1202. https://doi.org/10.3390/microorganisms9061202
33. Piggot P. J. Bacillus subtilis, Editor(s): Moselio Schaechter, Encyclopedia of Microbiology (Third Edition), Academic Press; 2009:45–56. https://doi.org/10.1006/rwgn.2001.0099
34. Kuebutornye F. K., et al. *In vitro* assessment of the probiotic characteristics of three *Bacillus* species from the gut of Nile tilapia, *Oreochromis niloticus*. *Probiot. Antimicro. Prot.* 2017; 12:412–424. https://doi.org/10.1007/s12602-019-09562-5
35. Khan F. F., Sohail A., Ghazanfar S., Ahmad A., Riaz A., Abbasi K. S., Ibrahim M. S., Uzair M., and Arshad M. Recent Innovations in Non-dairy Prebiotics and Probiotics: Physiological Potential, Applications, and Characterization. *Probiotics Antimicrob Proteins*. 2023. https://doi.org/10.1007/s12602-022-09983-9
36. Manhar A. K., et al. Cellulolytic potential of probiotic *Bacillus subtilis* AMS6 isolated from traditional fermented soybean (Churpi): An in vitro study with regards to application as an animal feed additive. *Microbiol. Res.* 2016; 186–187:62–70. https://doi.org/10.1016/j.micres.2016.03.004

37. Tirabunyanon M., and Tongwittaya N. Protection activity of a novel probiotic strain of Bacillus subtilis against Salmonella Enteritidis infection. *Res. Vet. Sci.* 2012; 93(1):74–81. https://doi.org/10.1016/j.rvsc.2011.08.008
38. Guo X., Chen D-D., Peng K-S., Cui Z-W., Zhang X-J., Li S., et al. Identification and characterization of *Bacillus subtilis* from grass carp (Ctenopharyngodon idellus) for use as probiotic additives in aquatic feed. *Fish Shellfish Immunol.* 2016; 52:74–84. https://doi.org/10.1016/j.fsi.2016.03.017
39. Cutting S. M. *Bacillus* probiotics. *Food Microbiol.* 2011; 28:214–220. https://doi.org/10.1016/j.fm.2010.03.007
40. Yasmin I., Saeed M., Khan W. A., Khaliq A., Chughtai M. F. J., Iqbal R., et al. *In vitro* probiotic potential and safety evaluation (hemolytic, cytotoxic activity) of *Bifidobacterium* strains isolated from raw camel milk. *Microorganisms.* 2020; 8:354. https://doi.org/10.3390/microorganisms8030354
41. Daneshazari R., Rabbani Khorasgani M., Hosseini-Abari A. and Kim J. H. *Bacillus subtilis* isolates from camel milk as probiotic candidates. *Sci. Rep.* 2023; 13(1):3387. https://doi.org/10.1038/s41598-023-30507-0
42. Fijan S. Microorganisms with claimed probiotic properties: An overview of recent literature. *Int. J. Environ. Res. Public Health.* 2014; 11:4745–4767. https://doi.org/10.3390/ijerph110504745
43. Ahire J. J., Kashikar M. S., and Madempudi R. S. Comparative accounts of probiotic properties of spore and vegetative cells of Bacillus clausii UBBC07 and in silico analysis of probiotic function. 3 Biotech. 2021; 11(3):116. https://doi.org/10.1007/s13205-021-02668-0
44. Shakira G., et al. (2018) Effect of indigenously isolated Saccharomyces cerevisiae probiotics on milk production, nutrient digestibility, blood chemistry and fecal microbiota in lactating dairy cows. *J. Anim. Plant Sci.* 2018; 28(2):407–420. https://api.semanticscholar.org/CorpusID:184482037. Accessed 22 Dec 2023.
45. Naeem M., et al. Screening of cattle gut associated Bacillus strains for their potential use as animal probiotic. *Indian J. Anim. Res.* 2018. https://doi.org/10.18805/ijar.B-948
46. Ripert G., Racedo S. M., Elie A. M., Jacquot C., Bressollier P. and Urdaci M. C. Secreted Compounds of the Probiotic Bacillus clausii Strain O/C Inhibit the Cytotoxic Effects Induced by Clostridium difficile and Bacillus cereus Toxins. *Antimicrob Agents Chemother*. 2016. https://doi.org/10.1128/AAC.02815-15
47. Nair A. S., and Dubhashi A. V. In-vitro transit tolerance of probiotic *Bacillus* species in human gastrointestinal tract. *Int. J. Sci. Res.* 2015; 5(6):1899–1902. https://doi.org/10.21275/v5i6.nov164343
48. Talebi S., Makhdoumi A., Bahreini M., Matin M. M., and Moradi H. S. Three novel Bacillus strains from a traditional lactofermented pickle as potential probiotics. *J. Appl. Microbiol.* 2018; 125(3):888–896. https://doi.org/10.1111/jam.13901
49. Araya M., Morelli L., Reid G., Sanders M. E. and Stanton C. *Joint FAO/WHO Working Group Report on Guidelines for the Evaluation of Probiotics in Food London*, Ontario. 2002. http://ftp.fao.org/es/esn/food/wgreport2.pdf.
50. Barbosa T. M., Serra C. R., La Ragione R. M., Woodward M. J., and Henriques A. O. Screening for Bacillus isolates in the broiler gastrointestinal tract. *Appl. Environ. Microbiol.* 2005; 71(2):968–978. https://doi.org/10.1128/AEM.71.2.968-978.2005
51. Lippolis R., Siciliano R., Mazzeo M., Abbrescia A., Gnoni A., Sardanelli A., and Papa S. Comparative secretome analysis of four isogenic Bacillus clausii probiotic strains. *Proteome Science.* 2013; 11(1):28. https://doi.org/10.1186/1477-5956-11-28

عزل وتوصيف وتقييم خصائص البروبيوتيك لبكتيريا *Bacillus clausii* المعزولة من براز الأطفال في إحدى المقاطعات الشمالية في فيتنام

نجوين كوينه آنه نجو[1*] ، شوان ثانه دام[2]، تيب خاك نجوين[2]، شين نجوك نجوين[3*] ، نهي دينه بوي[4*]

[1]مركز البحوث والأعمال الصيدلانية، كلية هاي دونج المركزية للصيدلة، 324 شارع نجوين لونج بانج، مدينة هاي دونج، فيتنام.

[2]كلية التكنولوجيا الحيوية، جامعة هانوي للصيدلة، 15-13 شارع لي ثانه تونج، مدينة هانوي، فيتنام.

[3]المعهد الوطني للتكنولوجيا الصيدلانية، جامعة هانوي للصيدلة، 15-13 شارع لي ثانه تونج، مدينة هانوي، فيتنام.

[4]جامعة فييت تري للصناعة، مدينة فييت تري، فيتنام

ملخص

Bacillus clausii هو بروبيوتيك بشري يستخدم على نطاق واسع في العديد من المنتجات التجارية؛ ومع ذلك، كان هناك بحث محدود حول عزل *Bacillus clausii* من مصادر متنوعة وتقييم خصائص البروبيوتيك .لأول مرة في هذه الدراسة، تم عزل سلالات *Bacillus clausii* وتقييمها من عينات البراز التي تم الحصول عليها من أطفال متطوعين أصحاء في مقاطعة شمال فيتنام .تم فحص الخصائص البيولوجية المتأصلة لسلالات *Bacillus clausii* المعزولة على وجه التحديد لاستكشاف تطبيقها المحتمل كبروبيوتيك .خضعت ثلاث عشرة مستعمرة للفحص من خلال التحليلات المورفولوجية والكيميائية الحيوية، جنبًا إلى جنب مع بروتين Maldi Tof MS. من بين هذه العزلات، تم تحديد *Bacillus* M23 و .M31 في فحص السلامة الأولي، أظهرت كلتا السلالات نشاطًا انحلاليًا سلبيًا .بالإضافة إلى ذلك، تم تحديد خصائص المختبر، مثل تكوين الجراثيم، ومقاومة الأحماض وأملاح الصفراء، ومقاومة الكائنات الحية الدقيقة المسببة للأمراض، وتقييم إنتاج الإنزيمات خارج الخلية، واختبار حساسية المضادات الحيوية لهذه السلالات، والتي تقع ضمن النطاق المرصود لسلالات البروبيوتيك الأخرى .كشف تسلسل جين 16S rRNA أن *Bacillus* M31 يشترك في تشابه بنسبة97 ٪ مع *Bacillus clausii* DSM 8716في قاعدة بيانات Genbank. تشير هذه النتائج إلى أن *Bacillus clausii* M31 يظهر وعدًا كمرشح بروبيوتيك، على الرغم من ضرورة إجراء المزيد من الدراسات المكثفة في المختبر / الجسم الحي للتحقق من فعاليته وسلامته.

الكلمات الدالة: العزل، التوصيف، *Bacillus clausii*، البروبيوتيك، براز الأطفال.

* ***المؤلف المراسل:***

نجوين كوينه آنه نجو qanhk63hup@gmail.com

شين نجوك نجوين: nhibd@vui.edu.vn

نهي دينه بوي: chiennn@hup.edu.vn

تاريخ استلام البحث 2024/02/28 وتاريخ قبوله للنشر 2024/05/18.

Rumex conglomeratus Murr. Grown Wild in Syria: Phytochemical Analysis and In Vitro Antioxidant Activities of Aerial Parts and Rhizomes Extracts

Rand AlKhoury*[1] and Racha AlKhatib[1]

[1]Faculty of Pharmacy, Damascus University, Damascus, Syrian Arab Republic.

ABSTRACT

Rumex conglomeratus Murr. (Polygonaceae), has been traditionally used to treat various conditions including skin-ailments, infections, constipation, and cancer. The medicinal importance of *Rumex* plants stems from their richness in many bioactive secondary metabolites. This study represents the first report on the chemical constituents and antioxidant activity of *Rumex conglomeratus* aerial parts and rhizomes extracts. The aqueous and ethanolic extracts were prepared and preliminary phytochemical screening tests were conducted. Total phenols, flavonoids, and anthraquinones contents were determined, along with the antioxidant activities, using colorimetric methods and a UV-visible spectrophotometer. The results revealed that *R. conglomeratus* is a rich source of secondary metabolites. Rhizomes ethanolic extract showed the highest content of phenols (502.55 ± 1.36 mg GAE/g DE) and anthraquinones (6.71 ± 0.106 mg RhE/g DE). It also exhibited the highest antioxidant activity as DPPH free radical scavengers (IC50 = 5.40 ± 0.380 mg/L), and as reducing agents in the FRAP assay (0.230 ± 0.004 at 200 mg/L), and TAC assay (321.41 ± 6.94 mg AAE/g DE). These findings suggest the potential use of *R. conglomeratus* as a potent antioxidant or even as a laxative agent. However, further research is essential to confirm the safety and efficacy, emphasizing the importance of continued exploration to isolate and identify the biologically active compounds.

Keywords: *Rumex Conglomeratus*; Polygonaceae; Phenols; Anthraquinones; Flavonoids; Antioxidant

1. INTRODUCTION

Oxidative stress has been involved in the pathophysiology of many life-threatening diseases, including cancer, cardiovascular diseases, neurodegenerative disorders, atherosclerosis, inflammation, and aging. Oxidative stress is a physiological condition that occurs when there is an imbalance between the body's antioxidant defense system and the production of free radicals, which are highly reactive atoms or molecules that have one or more unpaired electron[1,2]. The accumulation of free radicals, primarily reactive oxygen species (ROS) and reactive nitrogen species (RNS), can damage cellular structures such as proteins, lipids, and DNA, leading to oxidative damage in the body. This could occur under specific conditions, such as exposure to environmental toxins, chronic inflammation, or a poor diet[3].

Antioxidants are compounds that can neutralize free radicals and protect cells from oxidative damage. In recent years, there has been a significant attention on natural antioxidants derived from medicinal plants due to their safety and health benefits[4], and their high production of natural antioxidants such as vitamins C and E, carotenoids, and polyphenols[5,6].

Polyphenols, including phenolic acids, flavonoids, anthraquinones, tannins, stilbenes, and lignans, are the most significant class of secondary metabolites, exhibiting potent antioxidant activities through various mechanisms[7,4,8]. Anthraquinones, particularly phenolic-

[*]***Corresponding author: Rand AlKhoury***
rand1.alkhoury@damascusuniversity.edu.sy
Received: 29/02/2024 Accepted: 18/05/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2448

© 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

substituted anthraquinones, are abundant in the Polygonaceae plants, including *Rumex* L. species, and can inhibit the formation of free radicals or scavenge them through direct or indirect mechanisms[9,1].

The *Rumex* L. genus (Polygonaceae) is known for being rich in secondary metabolites, including anthraquinones, naphthalenes, flavonoids, tannins, stilbenes, terpenes, phenolic acids, and others[10]. The diverse chemical composition of *Rumex* L. species has made them a focal point for research and investigation of biological activities, including antioxidant, antibacterial, antiviral, antitumor, and antidiabetic properties[11,12]. *Rumex* L. plants have been utilized in traditional medicine to treat various health conditions such as skin ailments, bleeding, inflammation, constipation, and tumors[12,13]. Roots have been used as dyes due to their anthraquinones content. Leaves of many species are characterized by a sour taste, which is attributed to the presence of oxalic acid derivatives, and have been eaten as vegetables[12].

Rumex conglomeratus Murr., known as "clustered dock", and is called in Arabic "Hommaid or Hommaidah", is a wild edible plant spread widely in the northern temperate zone and Mediterranean region[14,15]. It has been used traditionally to treat scurvy, skin burns, rashes, eczema, and cancer. The leaves and aerial parts have been consumed as food[13,11].

To our knowledge, there are no previous studies on the chemical composition and biological activities of rhizomes and aerial parts of *R. conglomeratus*. Kilic *et al.* conducted a study on the aqueous extract of *R. conglomeratus* leaves collected from Turkey. They estimated the total phenols, flavonoids, carotenoids, and proline contents and evaluated the antioxidant activity[16]. Marelli *et al.* determined the total flavonoids content of 13 plants, including *R. conglomeratus*, and reported the presence of stigmasterol, ergostenol, and neophytadiene in the ethanolic extract of the leaves. They also evaluated the antioxidant activity[17]. Other studies have examined the antibacterial activity of ethanolic extracts from the stems, leaves, and roots, revealing that the roots exhibited the highest activity against *Staphylococcus aureus*[18], while the methanolic extract from the aerial parts showed the highest activity against *Moraxella catarrhalis*[19]. Therefore, this study aims to investigate the composition of secondary metabolites, total phenols, flavonoids, and anthraquinones content, and to evaluate the *In-vitro* antioxidant activity of aqueous and 95% ethanolic extracts prepared from the aerial parts and rhizomes of *R. conglomeratus* (Figure 1).

Figure 1: *Rumex conglomeratus* Murr. aerial parts and rhizome.

2. GENERAL EXPERIMENTAL:

2.1 Chemicals

All chemicals and reagents used in the study were of analytical grade. Ethanol absolute and Quercetin (from SIGMA-ALDRICH, Germany), Methanol Absolute and Ascorbic Acid (from Panreac, Spain), Gallic Acid, Sodium Carbonate anhydrous, Monosodium Phosphate, Disodium Phosphate, and Trisodium Phosphate (from AvonChem, United Kingdom), Folin-Ciocalteu reagent, Sulfuric Acid, and Magnesium Acetate (from Merck, Darmstadt, Germany), Sodium Acetate, Chloroform 99%, Aluminum Chloride, and Ferric Chloride (from Riedel-de Haen, Germany), Rhein (from MCE MedChemExpress USA), Concentrated Hydrochloric acid (from SHAMLAB, Syria), DPPH· 2,2-DiPhenyl-1-PicrylHydrazyl (from Tokyo Chemical Industry, Japan), Potassium Ferricyanide and Ammonium Molybdate (from Titan Biotech LTD, India) Trichloroacetic acid (from Scharlau, EU).

2.2 Plant material:

Plants of *Rumex conglomeratus* Murr. were collected in May 2022 from Ain Mneen in Damascus Countryside, Syria, at an altitude of 1200 m, with coordinates 33°38′32″N 36°17′52″E. The species was identified by Prof. Ahmad Jadouaa, Faculty of Agriculture, Aleppo University, based on the New Flora of Lebanon and Syria[14]. The muddy underground parts were rinsed with distilled water. The aerial parts and rhizomes were separated and dried in shade at room temperature for two weeks. Then, they were ground using an electric grinder to prepare the extracts.

2.3 Extraction:

Four crude extracts were prepared using Ultrasound-Assisted Extraction with two different solvents (distilled water and 95% ethanol) for each plant part. 20 grams of plant powder were extracted with 200 mL of solvent (distilled water or 95% ethanol) using Ultrasonic water bath cleaner (Skymen 040S 40kHz Industry Digital Heated Ultrasonic Cleaner, China) at a temperature of 55 – 60 °C for 30 minutes. Then, the extracts were filtered. The extraction process was repeated until the extract's color disappeared. All filtrates were collected and dried using a rotary evaporator (Zhengzhou Great Wall Rotary Evaporator R-1001-VN, China) under low pressure and 40°C for ethanolic extracts, and 50°C for aqueous extracts. The extraction yields were calculated using the following equation, then the four crude extracts were stored at 4°C for further analysis.

Extraction yield % = weight of the dried extract / weight of the dried powdered plant material x 100

2.4 Preliminary Phytochemical Screening:

Qualitative detection tests were done on each extract to investigate the presence of flavonoids, anthraquinones, tannins, coumarins, saponins, alkaloids, and cardiac glycosides.

2.4.1 Tests for flavonoids

Each extract was dissolved in 20% ethanol at 50°C for 3 to 5 minutes and then filtered.

Aluminum chloride test: 1 mL of aluminum chloride reagent was mixed with 1 mL of the extract. Flavonoids were identified by the blue or green fluorescence under UV light (365 nm)[20].

Shinoda test: 3 mL of the prepared extract were dried and then dissolved in 1 mL of absolute ethanol. Next, pieces of magnesium metal were added with a few drops of concentrated hydrochloric acid. The formation of a red, orange, or purple color indicates the presence of flavones or hydroxyflavones[21].

2.4.2 Tests for Anthraquinones[22]

Borntrager's test: Each extract was mixed with 1 mL of chloroform for 10 minutes then filtered. 2 mL of 10% NH3 solution were added slowly. In the presence of free anthraquinones, a red color appears in the upper ammonia layer.

Modified borntrager's test: the extract was put in 5 mL hydrochloric acid HCl 7% and 2.5 mL of 5% ferric chloride, and boiled under reflux for 15 minutes then filtered. The filtrate was shaken with 4 mL chloroform in a separating funnel and continued as in the borntrager's test. A red color will appear in the aqueous layer indicates the presence of anthraquinones glycosides.

2.4.3 Tests for tannins[23]

The extracts were dissolved in distilled water, and boiled for 5 minutes, and then filtered.

Ferric chloride test: A few drops of 10% ferric chloride were added to 1 mL of the filtrate. The formation of a dark green or dark blue color indicates the presence of tannins or polyphenols.

Gelatin precipitation test (specific): 5 mL of filtrate were mixed with 1 mL of diluted acetic acid, and a few drops of 1% gelatin solution with 10% sodium chloride were added. A white-abrownish precipitate will appear in the presence of tannins.

2.4.4 Tests for coumarins[24]

Fluorescence test: The extracts were boiled with distilled water for 3 minutes and then filtered. The filtrate was exposed to 365 nm UV light. A blue fluorescence indicates the presence of coumarins.

2.4.5 Tests for Saponins[22,25]

Foam test: Each extract was put in a test tube with 10 mL of hot water for a few minutes, then shaken vigorously for about 20 seconds. The formation of stable foam for at least 10 minutes indicates the presence of saponins.

2.4.6 Tests for alkaloids[23]

Precipitation tests: Each extract was dissolved in 3 mL of 10% HCl and 15 mL of water. The mixture was heated for 5 minutes and then filtered. A few drops of Mayer, Dragendorff, Wagner, and Hager reagents were added to the filtrate. The formation of white, orange, reddish-brown, and yellow precipitates respectively indicates the presence of alkaloids.

2.4.7 Tests for cardiac glycosides[23]

The extracts were boiled with 15 mL of 50% ethanol and 5 mL of lead acetate solution under reflux for 10 minutes, then filtered and cooled. The filtrate was shaken in a separating funnel with 5 mL of chloroform, and this process was repeated 3 times, then the chloroform layers were collected.

Keller Kiliani's test: 5 mL of the prepared extract were dried and dissolved in 1 mL of glacial acetic acid. One drop of 5% FeCl3 and 1 mL of concentrated H2SO4 were added. The formation of a reddish-brown color at the junction of the two liquid layers, and the bluish-green color at the upper acetic acid layer indicate the presence of deoxysugars in cardiac glycosides.

Kedde's test: 2 mL of the chloroform extract were evaporated, followed by the addition of 2 mL of the reagent (2% 3,5-dinitrobenzoic acid in 90% alcohol) and 1 mL of an alkaline solution (20% sodium hydroxide solution). A purple color is produced in the presence of β-unsaturated-o-lactones (cardenolides).

2.5 Total Phenolic Content (TPC) in R. conglomeratus extracts:

2.5.1 Gallic acid standard and extracts preparation

A series of dilutions ranging from 0 to 500 mg/L was made from gallic acid standard solution in distilled water, and the extracts were prepared at a concentration of (600 mg/L).

2.5.2 Determination of total phenols

The total phenols content was determined using the Folin-Ciocalteu Reagent (FCR) method as described by Agha and Hussain[26], with slight modifications. Briefly, 40 µl of sample (Gallic acid or extract), 2 mL of distilled water, and 200 µl of 10% FCR were mixed. After 5 minutes, 600 µl of 10% Na2CO3 solution were added. Reaction tubes were covered, vortexed, and placed in the dark for 2 hours at room temperature. The absorbance reading of each sample was measured using a UV-visible spectrophotometer (Model: T80+, PG instrument Ltd, United Kingdom) at the maximum absorbance wavelength, λ_{max} = 762 nm. The blank was prepared with 2 mL of distilled water. The total phenolic content was calculated using linear equation of the calibration curve for Gallic acid ($y= 0.0012x + 0.0265$) ($R^2 = 0.9953$). All results were expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of the Dried Extract (DE).

2.6 ***Total Flavonoids content (TFC) in R. conglomeratus extracts:***

2.6.1 Quercetin standard and extracts preparation

A series of dilutions ranging from 0 to 50 mg/L was made from quercetin standard solution in methanol, and

the extracts were prepared at a concentration of 200 mg/L

2.6.2 Determination of total Flavonoids

The total flavonoids content was measured using the aluminum chloride colorimetric method described by Khatib and Al-Makky[27], with minor modifications. Briefly, 2 mL of the sample (quercetin or extract), 100 µl of 1 M sodium acetate, 100 µl of 10% methanolic aluminum chloride solution, and 2.8 mL of distilled water were mixed. Reaction tubes were covered, vortexed, and incubated in the dark at room temperature. The reaction will produce a yellow aluminum-flavonoid complex. After 30 minutes, the absorbance reading of each sample was measured using a UV-visible spectrophotometer at the maximum absorbance wavelength, λ_{max} **= 429 nm**. The blank was prepared with 2 mL of absolute methanol. The total flavonoids content was calculated using the calibration curve equation for quercetin **(y= 0.0274x + 0.0154) (R^2 = 0.9967)**. All results were expressed as milligrams of Quercetin Equivalents (QE) per 1 gram of the Dried Extract (DE).

2.7 ***Total Anthraquinones content (TAnC) in R. conglomeratus extracts:***

The total anthraquinones content was measured using the colorimetric method described by Sakulpanich and Gritsanapan[28]. The method depends on Borntrager's reaction principle, which is based on the ability of free anthraquinones (Aglycones) to react with alkali and form ions that are visibly pinkish-red colored[29].

2.7.1 Rhein standard

A series of seven dilutions ranging from 0 to 8.64 mg/L was made from Rhein standard solution in 0.5% magnesium acetate solution in methanol forming pinkish-red colors.

2.7.2 Extracts preparation

50 mg of the extract containing anthraquinones (Aglycones + glycosides) was accurately weighed, and 10 mL of distilled water were added. The mixture was stirred, weighed, and then refluxed on a water bath for 15 minutes. The flask was allowed to cool, weighed, and then adjusted to the original weight with distilled water. 20 mL of 10.5% FeCl3 and 1 mL of concentrated HCl were added (to break O-glycosides and C-glycosides bonds). The mixture was weighed and refluxed for 20 minutes, then allowed to cool and adjusted to the original weight, anthraquinones turned into the free aglycone form. The mixture was shaken with 25 mL of chloroform in a separating funnel, and the chloroform layer was collected, the extraction was repeated three times. The chloroform layers were filtered and transferred to a 100 mL volumetric flask, and the volume was adjusted with chloroform. 25 mL of the chloroform extract were evaporated and the residue was dissolved in 10 mL of 0.5% magnesium acetate in methanol (Alkali), resulting in a pinkish-red color.

2.7.3 Determination of total Anthraquinones:

The absorbance of the resulting pinkish-red color was measured using a UV-visible spectrophotometer at 517 nm. The total anthraquinones content (represents aglycones + glycosides forms) was calculated from the calibration curve equation for Rhein **(y= 0.0405x + 0.0038) (R^2 = 0.9976)**. All results were expressed as milligrams of Rhein Equivalents (RhE) per gram of the Dried Extract (DE).

2.8 ***In vitro antioxidant activity assessment of R. conglomeratus extracts:***

2.8.1 DPPH• free radicals scavenging activity:

The free radical scavenging activity of *R. conglomeratus* extracts was measured using the method of Kilic et al.[16], with some modifications. The extracts were prepared in five concentrations (5, 10, 25, 50, 100) mg/L from the aerial parts aqueous extract, and (2, 5, 10, 25, 50) mg/L from the other extracts. A series of six concentrations (0.5 to 10 mg/L) of gallic acid solution in ethanol was made. DPPH solution in ethanol (45 mg/L) was prepared. The reaction was done by adding 300 µl of the sample (gallic acid or extract) to 1500 µl of DPPH solution, and control was made by adding 300 µl of ethanol to 1500 µl of DPPH solution. The reaction was then incubated in the dark at room temperature. After 30 minutes, the absorbance was measured at 517 nm. The decreasing in absorbance indicates

greater radical scavenging activity. The percentage of DPPH free radical scavenging activity (RSA%) was calculated using the following equation, and the inhibitory concentration of 50% of DPPH free radicals (IC50 mg/L) was calculated for extracts and gallic acid from the graphs of the relationship between sample concentration and RSA%. The lowest IC50 value indicates the highest radical scavenging activity.

Radical Scavenging Activity % (RSA%) = 100 x (Ac – As) / Ac
Ac: the absorbance of control, As: the absorbance of sample

2.8.2. Ferric Reducing Antioxidant Power (FRAP Assay)

The reducing power of the *R. conglomeratus* extracts was determined according to the method described by Khatoon et al.[30], with slight modifications. Five different concentrations of extracts and ascorbic acid standard in methanol (25-200 mg/L) were prepared. 1 mL of the sample (extract or ascorbic acid), 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide (1 %) were mixed. The mixture was then incubated at 50ºC for 20 minutes. Next, 2.5 mL of 10 % trichloroacetic acid was added. After 10 minutes, the mixture was filtered. 1.8 mL of the filtrate were mixed with 1.8 mL of distilled water and 0.5 mL of 0.1% FeCl3. The absorbance was measured at **700 nm**. The increasing absorbance demonstrates the Fe^{+3} reducing power and antioxidant capacity of the sample. All measurements were done in triplicate and results were expressed as the mean ± standard deviation.

2.8.3. **Phosphomolybdate assay (Total Antioxidant Capacity TAC)**

The total antioxidant capacity was evaluated following the phosphomolybdate method described by Khatoon et al.[30], using ascorbic acid as a standard. Five different concentrations (25, 50, 100, 150, 200 mg/L) were prepared from extracts and ascorbic acid. 0.3 mL of the sample (extract or ascorbic acid) were mixed with 3 mL of the reagent (ammonium molybdate 0.4 mM, sulfuric acid 0.6 M, and trisodium phosphate 28 mM). The reaction tubes were incubated at 95°C for 90 minutes and then cooled to room temperature. In the presence of antioxidants, Mo (VI) is reduced to Mo (V) and forms a green-colored phosphomolybdenum (V) complex. The absorbance readings were measured at 695 nm. The TAC was calculated using the following equation of the ascorbic acid calibration curve ($y= 0.0043x + 0.1376$) ($R^2 = 0.9942$).

2.9 ***Statistical analysis:***

All measurements were done in triplicate. Results were expressed as the mean ± standard deviation. Correlation coefficient (r^2) between TPC, TFC, TAnC and the antioxidant activities were calculated using Microsoft Excel (2019) software

3. RESULTS

3.1 Yield of extraction:

The extraction resulted in four crude extracts, APA: Aerial parts aqueous extract, APE: Aerial parts ethanolic extract, RA: Rhizomes Aqueous extract, RE: Rhizomes Ethanolic extract. Table 1 shows the percentage yield of each prepared extract. The RA extract had the highest yield (28.5%), while the APE extract had the lowest yield (20.4%).

Table 1: Extraction yields of *R. conglomeratus* extracts*:*

Plant part	**Solvent of extraction**	**Yield%**
Aerial parts	Distilled water	23.35
	95% Ethanol	20.4
Rhizomes	Distilled water	28.5
	95% Ethanol	26

3.2 Preliminary Phytochemical Screening:

The results of the preliminary investigation for secondary metabolites are shown in Table 2. The (+) mark indicates a positive test result, while the (–) mark indicates a negative result. All extracts contain Flavonoids, Anthraquinones, Tannins, Coumarins, and Saponins.

Table 2: the results of preliminary investigation tests for secondary metabolites in *R. conglomeratus* extracts

Secondary metabolites	Test name	APA[1] extract	APE[2] extract	RA[3] extract	RE[4] extract
Flavonoids	Aluminum chloride	+	+	+	+
	Shinoda	+	+	+	+
Anthraquinones	Borntrager	+	+	+	+
	Modified Borntrager	+	+	+	+
Tannins	Ferric chloride	+	+	+	+
	gelatin	+	+	+	+
Coumarins	Fluorescence	+	+	+	+
Saponins	Foam	+	+	+	+
Alkaloids	Dragendorff	–	–	–	–
	Mayer	–	–	–	–
	Hager	–	–	–	–
	Wagner	–	–	–	–
Cardiac glycosides	Keller-Kiliani	–	–	–	–
	Kedde	–	–	–	–

[1]*aerial parts aqueous extract,* [2]*aerial parts ethanolic extract,* [3]*rhizomes aqueous extract,* [4]*rhizomes ethanolic extract.*

3.3 Total phenolic content

The total phenolic contents of R. conglomeratus extracts ranged between 279.86 ± 3.02 GAE/g DE and 502.55 ± 1.36 GAE/g DE (Table 3). The rhizomes' ethanolic extract had the highest phenolic content followed by RA, APE, APA extracts respectively.

3.4 Total flavonoids content

The aerial parts' ethanolic extract of *R. conglomeratus* had the highest flavonoids content (46.82 ± 0.394) mg QE/g DE, while the lowest flavonoids content was found in the rhizomes' aqueous extract (8.45 ± 0.504) mg QE/g DE (Table 3).

3.5 Total Anthraquinones content

The total anthraquinones contents of *R. conglomeratus* extracts ranged from 1.26 ± 0.093 mg RhE/g DE, to 6.71 ± 0.106 mg RhE/g DE. The rhizomes' ethanolic extract had the highest anthraquinones content, while the aerial parts' aqueous extract had the lowest content (Table 3)

Table 3: Total phenols (TPC), flavonoids (TFC), and Anthraquinones (TAnC) content in *R. conglomeratus* extracts

***R. conglomeratus* extract**	TPC mg GAE[1]/g DE[2]	TFC mg QE[3]/g DE	TAnC mg RhE[4]/g DE
APA	279.86 ± 3.02	28.54 ± 0.661	1.26 ± 0.093
APE	381.71 ± 2.85	46.82 ± 0.394	3.35 ± 0.062
RA	431.10 ± 1.57	8.44 ± 0.504	4.55 ± 0.125
RE	502.55 ± 1.36	12.30 ± 0.986	6.71 ± 0.106
[1]gallic acid equivalent, [2]dry extract , [3]quercetin equivalent, [4]rhein equivalent			

3.6 In vitro antioxidant activity assessment

3.6.1 DPPH scavenging activity:

The free radicals scavenging (RSA%) for each extract and gallic acid are shown in Figure 2. The IC50 values for gallic acid and *R. conglomeratus* extracts are also mentioned in Table 4. The most effective extract was the rhizomes' ethanolic extract, with an IC50 = 5.40 ± 0.380 mg/L. While aerial parts' aqueous extract had the lowest efficacy, with an IC50= 43.36 ± 0.474 mg/L.

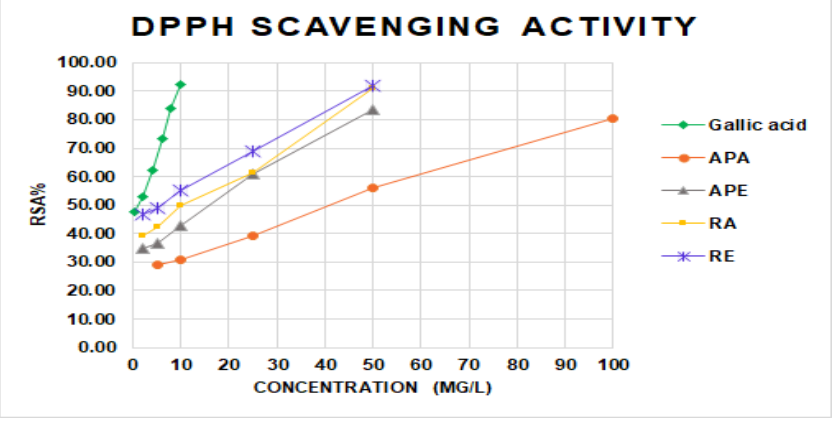


Figure 2: DPPH free radical scavenging activity (RSA%) of *R. conglomeratus* extracts and gallic acid

Table 4: RSA% and IC50 values of *R. conglomeratus* extracts and gallic acid standard in DPPH assay

***R. conglomeratus* extract**	**RSA% after 30 minutes at C = 10 mg/L**	**RSA% after 30 minutes at C = 50 mg/L**	**IC50 (mg/L)**
APA	30.90 ± 0.22	80.27 ± 0.32	43.36 ± 0.474
APE	42.89 ± 0.40	83.57 ± 0.54	16.80 ± 0.385
RA	49.81 ± 0.19	91.26 ± 0.19	11.97 ± 0.189
RE	55.08 ± 0.15	91.87 ± 0.26	5.40 ± 0.380
Gallic acid	92.44 ± 0.18	—	1.23 ± 0.008

3.6.2 FRAP Assay

The ferric reducing power of the extracts and ascorbic acid was concentration-dependent (Figure 3). At a concentration of 200 mg/L, the ethanolic extract of rhizomes exhibited the highest reducing power, with the highest absorbance (0.230 ± 0.004), although lower than that of ascorbic acid (0.923 ± 0.003). The aerial parts aqueous extract had the lowest reducing power (0.198 ± 0.003) (Table 5).

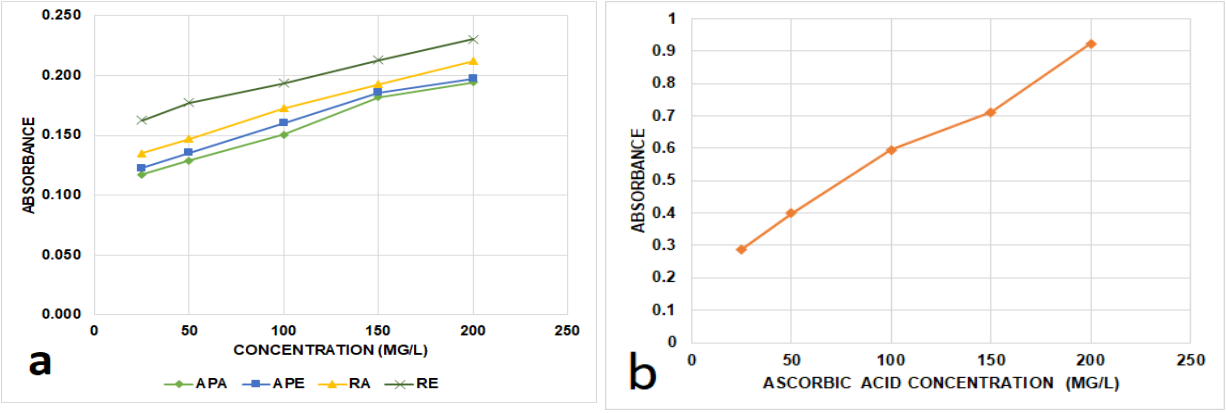


Figure 3: Ferric reducing power of (a) *R. conglomeratus* extracts, and (b) Ascorbic Acid standard

Table 5: Absorbance readings of R. conglomeratus extracts and Ascorbic Acid standard in FRAP Assay

Concentration (mg/L)	APA	APE	RA	RE	Ascorbic Acid
25	0.117 ± 0.003	0.123 ± 0.003	0.135 ± 0.003	0.163 ± 0.003	0.288 ± 0.001
50	0.129 ± 0.002	0.136 ± 0.003	0.147 ± 0.003	0.177 ± 0.004	0.400 ± 0.001
100	0.151 ± 0.001	0.160 ± 0.002	0.173 ± 0.002	0.194 ± 0.005	0.596 ± 0.002
150	0.182 ± 0.003	0.186 ± 0.001	0.193 ± 0.004	0.213 ± 0.004	0.711 ± 0.001
200	0.194 ± 0.002	0.198 ± 0.003	0.212 ± 0.002	0.230 ± 0.004	0.923 ± 0.003

3.6.3 Phosphomolybdate assay (Total Antioxidant Capacity):

The total antioxidant capacity of the *R. conglomeratus* extracts was expressed as the number of ascorbic acid equivalents (Table 6). The rhizomes ethanolic extracts had the highest antioxidant capacity (321.41 ± 6.94 mg AAE/g DE), while aerial parts aqueous extract had the lowest total antioxidant capacity (39.75 ± 3.16 mg AAE/g DE).

Table 6: Total antioxidant capacity of *R. conglomeratus* extracts in phosphomolybdate assay

R. conglomeratus extract	Total Antioxidant Capacity (mg AAE/g DE)
APA	39.75 ± 3.16
APE	174.33 ± 5.32
RA	264.56 ± 3.31
RE	321.41 ± 6.94

3.7 Statistical analysis:

Table 7 shows the correlation coefficients between TPC, TFC, TAnC and the antioxidant activities of the studied extracts.

Table 7: Correlation coefficients

	Correlation coefficient (r^2)		
	TPC	**TFC**	**TAnC**
IC50 (DPPH)	0.9263	0.1411	0.8671
FRAP	0.8541	0.5286	0.9108
TAC	0.9838	0.3355	0.955

DISCUSSION:

In this study, the extracts from aerial parts and rhizomes of *R. conglomeratus* Murr. were analyzed for the first time. The approach involved investigating the presence/absence of some secondary metabolites, determining the total content of phenols, flavonoids, and anthraquinones, and evaluating the antioxidant activity using three in vitro antioxidant assays: DPPH, FRAP, and TAC.

3.8 Yields of extraction

The four extracts prepared from *R. conglomeratus* aerial parts and rhizomes showed different extraction yields. The rhizomes extracts had higher yields than the aerial parts extracts, indicating that rhizomes contain a higher percentage of metabolites. The aqueous extracts of each plant part gave a higher extraction yield than ethanolic extracts, because the extraction solvent significantly affects the quality of the extracted compounds[31]. This suggests that the extracted substances are of a high polarity and predominantly present in the form of salts or glycosides which tend to dissolve more in water.

3.9 Phytochemical analysis

Preliminary phytochemical screening reactions revealed that all studied extracts of *R. conglomeratus* are rich in various secondary metabolites including, phenols, flavonoids, anthraquinones, tannins, coumarins, and saponins. The positive results of Shinoda's test indicated the presence of flavones and hydroxyflavones compounds[32], while the positive results of Borntrager's and modified Borntrager's tests indicated the presence of anthraquinones in both free aglycone and glycoside forms. While alkaloids and cardiac glycosides were absent in all extracts. Our results were similar to those reported by Mekonnen *et al.*[33] on ***R. abyssinicus*** rhizomes in Ethiopia, Gebrie *et al*.[34] on ***R. steudelii*** roots in Ethiopia, and Jaradat *et al.*[35] on ***R. rothschildianus*** leaves in Palestine. Whereas Ammar *et al*.[36] (Egypt) reported the absence of saponins and coumarins in the aerial parts of ***R. vesicarius*** and ***R. pictus***, and Hafaz *et al*. (Egypt) reported the presence of alkaloids in roots and shoots of ***R. dentatus***, ***R. pictus***, and ***R. vesicarius***, and the absence of coumarins in the roots of the three mentioned species[37].

The quantitative estimation of phenols, flavonoids, and anthraquinones revealed that the highest levels of total phenols and anthraquinones contents were observed in the rhizomes ethanolic extract (RE). Whereas, aerial parts ethanolic extracts (APE) had the highest content of total flavonoids. These results indicated that rhizomes contain higher amounts of phenols and anthraquinones than the aerial parts. While the aerial parts have a higher flavonoids content, which could be explained by the fact that flavonoids, especially hydroxyflavones, are mostly produced in flowers and vegetative organs to attract pollinators, or as a defense against environmental conditions[38]. We can also notice that the phenolic content was much higher than flavonoids and anthraquinones contents in all extracts. This could be due to the presence

of other phenolic metabolites such as tannins, stilbenes, lignans, and phenolic acids. From another perspective, the elevated phenols content could be related to the Folin-Ciocalteu method used, which depends on the reduction of the FC reagent by the phenolic compounds. However, the presence of reductants that contain hydroxyl groups other than phenols, such as reducing sugars, ascorbic acid, or proteins, will interfere and reduce the reagent as well. Therefore, this method might have overestimated the phenolic content in the studied extracts[39,40].

Compared with literature, previous studies on ***R. conglomeratus*** are very limited, Kilic *et al*. (Turkey) reported a lower TPC (43 mg/g dry extract) in the aqueous extract of leaves, but a higher TFC (119 mg/g)[16], and Marelli *et al.* (Italy) reported a lower TFC (15.5 mg/g) in leaves ethanolic extract[17]. Comparing with other *Rumex* species, a study on ***R. crispus*** (South Africa) confirmed the higher TPC in roots and ethanolic extracts, consistent with the findings of this study. The roots' ethanolic extract had a lower TPC (211.71 ± 9.65 mg GAE/g DE) but a higher TFC (45.19 ± 1.44 mg QE/g DE) compared to this study[41]. The roots' methanolic extract of ***R. roseus*** (Tunisia) also showed a lower TFC (10.81 mg QE/g DE) compared to the aerial parts (44.28 mg QE/g DE) and to the finding of this study[42]. TPC of the studied *R. conglomeratus* exceeded that of many other previously studied species, such as roots' ethanolic extract of ***R. crispus*** (Korea), (21.84 mg GAE/g/ DE)[43], aerial parts' methanolic extract of ***R. vesicarius*** (Algeria) (43.28 mg GAE/g/ DE)[44], and leaves and roots ethanolic extracts of ***R. dentatus*** (Egypt) (50.1 mg GAE/g/ DE) and (101.6 mg GAE/g/ DE) respectively[45].

Anthraquinones have potent biological activities[46], the laxative and digestion-enhancing effects are the most known for these compounds. Recent studies have evaluated the effectiveness of naturally occurring anthraquinones as antioxidant agents, and against coronavirus based on their antiviral and immune-boosting properties, in the context of finding new effective and safe drugs for the COVID-19 pandemic[47]. In this study, the total anthraquinones content in extracts of *R. conglomeratus* was determined. The rhizomes ethanolic extract had the highest anthraquinones content, which represents **0.67%** of the dry extract's weight, and about **0.18%** of the dry rhizome's weight. This is consistent with previous studies that anthraquinones are concentrated mainly in the underground parts of *Rumex* plants[48]. Eom *et al*.[43] reported the TAnC content of ***R. crispus*** roots ethanolic extract (22.97 ± 0.026 mg/g DE) higher than *R. conglomeratus* extracts, and Seitimova *et al*. reported a higher TAnC of ***R. tianschanicus*** roots ethanolic extract (1.77%)[49]. Litvinenko & Muzychkina reported the TAnC of the roots of 7 *Rumex* species, the highest content was for ***R. aquaticus*** (2.13%), and the lowest in ***R. thyrsiflorus*** (0.71%)[50].

Differences in qualitative and quantitative phytochemical analysis between different species could be attributed to several factors including genetic makeup, environmental conditions, and climate factors such as temperature, humidity, exposure to ultraviolet radiation, drought and soil type. These factors can affect the plant's requirements and the synthesis rate of secondary metabolites. The extraction method, solvent, plant part under study, calibration method, and standards also play crucial roles in the outcomes of these studies[51,52].

3.10 In vitro antioxidant activity

In vitro antioxidant activity assays revealed that all extracts exhibit free radical scavenging activity and reducing power, which are positively correlated with extract's concentration. The rhizomes' ethanolic extract showed the highest antioxidant activity in all applied assays, although it was lower than the standards. The antioxidant activities can be attributed mainly to the secondary metabolites, especially the predominance of polyphenols. The antioxidant activity increased with the ascendant contents of phenols and anthraquinones with a high correlation (refer to Table 7), suggesting that the effectiveness is highly related to them. The increase in flavonoids content in each plant part has been accompanied

by an increase in the antioxidant activity as well. Phenols, flavonoids, and anthraquinones are potent secondary metabolites that are recognized for their strong antioxidant activity through various mechanisms, they can directly scavenge free radicals or act as reducing agents, hydrogen or electron donors, and/or metal ions chelators[53]. The nature of bioactive compounds and their structures greatly affect the antioxidant activity[3], the increasing number of hydroxyl groups and the attachment of side chains to the aromatic rings give a higher antioxidant capacity of phenolic compounds[53]. Therefore, further research and isolation of chemical compounds responsible for the activity are highly recommended to confirm the results.

According to the findings of this study, *R. conglomeratus* extracts, especially the rhizomes, are rich in natural antioxidants, and could be used to prevent the body from serious health conditions related to oxidative stress. They can also be used in food industry as food preservatives, or as laxatives and digestion enhancers due to the anthraquinones content. The daily dose of anthraquinones as laxatives, ranges between 15 to 30 mg/day[54,55] . Therefore, it can be suggested to use *R. conglomeratus* rhizomes as laxatives at a dose ranging between (9 – 17) grams per day, or to use the dried ethanolic extract at a dose of (2 – 4.5) grams per day, one to three times a week at most. However, subsequent studies must be conducted to confirm the safety and effectiveness of the drug within this framework or other medicinal applications.

4. CONCLUSIONS

This study represents the first report on the chemical constituents and antioxidant activity of the aerial parts and rhizomes of *Rumex conglomeratus*. The findings revealed that *Rumex conglomeratus*, particularly the rhizome, is a reliable source of secondary metabolites and rich in phenols, flavonoids, and anthraquinones, with strong antioxidant activity as free radical scavengers and reducing agents. The rhizomes ethanolic extract had the highest antioxidant activity, which was highly correlated to the content of phenols and anthraquinones. Therefore, the results suggest using *R. conglomeratus* extracts as potent antioxidants that could protect the body from serious health conditions, or even as laxative agents or digestion-enhancers. However, further research is essential to confirm their safety and efficacy, emphasizing the importance of continued exploration into isolating and identifying biologically active compounds using a variety of chromatographic methods such as HPLC and LC-MS.

Acknowledgements

Authors are grateful to Dr. Bassam AlMeslmani for his help to get materials necessary to this study.

Funding: This research was funded by Damascus University – funder No. 501100020595.

Conflict of interest:

The authors declare no conflict of interest.

REFERENCES

1. Zhao L. and Zheng L. A Review on Bioactive Anthraquinone and Derivatives as the Regulators for ROS. *Molecules.* 2023; 28: 1–23.
2. Liguori I., Russo G., Curcio F., et al. Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*. 2018; 2018: 757–772.
3. Shahidi F. and Zhong Y. Measurement of antioxidant activity. *Journal of Functional Foods.* 2015; 18: 757–781.
4. Llauradó Maury G., Méndez Rodríguez D., Hendrix S., et al. Antioxidants in plants: A valorization potential emphasizing the need for the conservation of plant biodiversity in Cuba. *Antioxidants.* 2020; 9: 1–36.

5. Ammar S., Abidi J., Luca S. V., et al. Untargeted metabolite profiling and phytochemical analysis based on RP-HPLC-DAD-QTOF-MS and MS / MS for discovering new bioactive compounds in Rumex algeriensis flowers and stems. *Phytochemical Analysis*. 2020; 2020: 1–20.
6. Pizzino G., Irrera N., Cucinotta M., et al. Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*. 2017; 2017: 1–13.
7. Al-Assaf I., Khazem M. Antioxidant Activity of Total phenols and Flavonoids extracted from Echinops polyceras roots grown in Syria. *Iraqi Journal of Pharmaceutical Sciences*. 2021; 30: 261–268.
8. Abu-darwish D., Shibli R., Al-abdallat A. M. Phenolic Compounds and Antioxidant Activity of Chiliadenus montanus (Vhal .) Brullo . grown in vitro. *Jordan Journal of Pharmaceutical Sciences*. 2024; 17: 611–628.
9. Li Y., Jiang J. Health functions and structure–activity relationships of natural anthraquinones from plants. *Food & Function*. 2018; 2018: 6063–6080.
10. Shafiq N., Saleem M., Kousar S., et al. Investigation of Genus Rumex for Their Biologically Active Constituents. *RJLBPCS*. 2017; 2: 148–163.
11. Vasas A., Orbán-Gyapai O., Hohmann J. The Genus Rumex: Review of traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*. 2015; 9: 1–94.
12. Li J-J., Li Y-X., Li N., et al. The genus Rumex (Polygonaceae): an ethnobotanical, phytochemical and pharmacological review. *Natural Products and Bioprospecting*. 2022; 12: 1–29.
13. Sarı F., Koçyiğit M. Ethnobotanical Usages of the Turkish Rumex Taxa. *Turkish Journal of Bioscience and Collections*. 2021; 5: 123–140.
14. MOUTERDE P. *NOUVELLE FLORE DU LIBAN ET DE LA SYRIE*. TOME PREMI. BEYROUTH: ÉDITIONS DE L'IMPRIMERIE CATHOLIQUE, 1966. Epub ahead of print 1966. DOI: 10.4000/books.editionsmsh.2669.
15. Keskin M., Severoğlu Z. The genus of Rumex (Polygonaceae) in Istanbul and the new check-list of Polygonaceae in Türkiye. *Frontiers in Life Sciences and Related Technologies*. 2023; 4: 13–19.
16. Kılıç I., Yeşiloğlu Y., Bayrak Y., et al. Antioxidant Activity of Rumex conglomeratus P . Collected from Turkey. *Asian J Chem*. 2013; 25. Epub ahead of print. DOI: 10.14233/ajchem.2013.15130.
17. Marrelli M., Cristaldi B., Menichini F., et al. Inhibitory effects of wild dietary plants on lipid peroxidation and on the proliferation of human cancer cells. *Food and Chemical Toxicology*. 2015; 86: 16–24.
18. Ramírez A., Luz S., Díaz B., et al. Actividad Antibacteriana De Extractos Y Fracciones Del Ruibarbo (Rumex conglomeratus). *Scientia Et Technica*. 2007; 13: 397–400.
19. Orbán-Gyapai O., Liktor-Busa E., Kúsz N., et al. Antibacterial screening of Rumex species native to the Carpathian Basin and bioactivity-guided isolation of compounds from Rumex aquaticus. *Fitote*. 2017. Epub ahead of print.
    DOI: 10.1016/j.fitote.2017.03.009.
20. Ghannam M., Shammaa E., Ali A. Determining the quality of the powders of Xanthium Strumarium and Xanthium Spinosum by microscopic examination and preliminary tests. *SN Applied Sciences*. 2020; 2:1–12.
21. Mondal S., Rahaman S.T. Flavonoids : A vital resource in healthcare and medicine. *Pharmacy & Pharmacology International Journal*. 2020; 8:91–104.
22. Nortjie E., Basitere M., Moyo D., et al. Extraction Methods , Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles : A Review. *Plants*. 2022; 11:1–17.
23. Shaikh J.R., Patil M. Qualitative tests for preliminary phytochemical screening : An overview. *International Journal of Chemical Studies*. 2020; 8:603–608.

24. Zohra S.F., Meriem B., Samira S., et al. Phytochemical Screening and identification of some compounds from Mallow. *Journal of Natural Products and Plant Resources.* 2012; 2:512–516.
25. Dilshad R., Batool R. Antibacterial and Antioxidant Potential of Ziziphus jujube , Fagonia Arabica , Mallotus phillipensis and Hemidesmus Indicus. *Jordan Journal of Pharmaceutical Sciences*. 2022; 15:413–427.
26. Hussain H.G., Agha M.I.H. Evaluation of the thrombolytic activity of the methanolic extract of Melia azedarach fruits and leaves in vitro. *Journal of Advanced Biotechnology and Experimental Therapeutics*. 2022; 5: 634–641.
27. Khatib R., Al-Makky K. Anti-Oxidant and Anti-Bacterial Activities of Sinapis alba L. (Leaves, Flowers and Fruits) Grown in Syria. *Bulletin of Pharmaceutical Sciences Assiut University* 2021; 44: 339–347.
28. Sakulpanich A., Gritsanapan W. Extraction Method for High Content of Anthraquinones From Cassia Fistula Pods. *J Health Res.* 2008; 22:167–172.
29. Humadi S., Obaid A. *Anthroaquinone Glycosides (Introduction and Extraction) Lab*. Karblaa - Iraq: AL ZAHARAWI UNIVERSITY COLLEGE DEPARTMENT OF PHARMACY, 2019.
30. Khatoon M., Islam E., Islam R., et al. Estimation of total phenol and in vitro antioxidant activity of Albizia procera leaves. *BMC Research Notes*. 2013; 6:1–7.
31. Truong D., Nguyen D.H., Ta N.T.A., et al. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. *Journal of Food Quality*. 2019; 2019:1–9.
32. María R., Shirley M., Xavier C., et al. Preliminary Phytochemical Screening, Total Phenolic Content and Antibacterial Activity of Thirteen Native Species from Guayas Province Ecuador. *Journal of King Saud University - Science*. 2017. Epub ahead of print. DOI: 10.1016/j.jksus.2017.03.009.
33. Mekonnen A., Desta W. Comparative study of the antioxidant and antibacterial activities of Rumex abyssinicus with commercially available Zingiber officinale and Curcuma longa in Bahir Dar city , Ethiopia. *Chemical and Biological Technologies in Agriculture*. 2021; 8:1–11.
34. Gebrie E., Makonnen E., Debella A., et al. Phytochemical screening and pharmacological evaluations for the antifertility effect of the methanolic root extract of Rumex steudelii. *Journal of Ethnopharmacology*. 2005; 96:139–143.
35. Jaradat N., Hawash M. anti-obesity activities of Rumex rothschildianus Aarons . extracts. *BMC Complementary Medicine and Therapies.* 2021; 21:1–11.
36. Ammar N., Ayoub N., El-Ahmady S., et al. Phytochemical and Cytotoxic Studies of Rumex pictus Forssk. and Rumex vesicarius L. (Family Polygonaceae), Growing in Egypt. *European Journal of Medicinal Plants.* 2015; 10:1–13.
37. Hafaz M.F., Soliman H.M., Abbas M.A., et al. Potential assessment of rumex spp. As a source of bioactive compounds and biological activity. *Biointerface Research in Applied Chemistry*. 2022; 12:1824–1834.
38. del Valle J.C., Buide M.L., Casimiro-Soriguer I., et al. On flavonoid accumulation in different plant parts: variation patterns among individuals and populations in the shore campion (Silene littorea). *Frontiers in Plant Science*. 2015; 6:1–13.
39. Pérez M., Dominguez-López I., Lamuela-Raventós R.M. The Chemistry Behind the Folin-Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. *Journal of Agricultural and Food Chemistry.* 2023; 71:17543–17553.
40. Lawag I.L., Nolden E.S., Schaper A.A.M., et al. A Modified Folin-Ciocalteu Assay for the Determination of Total Phenolics Content in Honey. *Applied Sciences (Switzerland).* 2023; 13:1–17.

41. Idris O.A., Wintola O.A., Afolayan A.J. Asian Paci fi c Journal of Tropical Biomedicine. *Asian Pacific Journal of Tropical Biomedicine.* 2017; 7:1071–1078.
42. Chelly M., Chelly S., Occhiuto C., et al. Comparison of Phytochemical Profile and Bioproperties of Methanolic Extracts from Different Parts of Tunisian Rumex roseus. *Chemistry & Biodiversity.* 2021; 18:1–11.
43. Eom T., Kim E., Kim J. In Vitro Antioxidant, Antiinflammation, and Anticancer Activities and Anthraquinone Content from Rumex crispus Root Extract and Fractions. *Antioxidants.* 2020; 9:1–13.
44. Beddou F., Bekhechi C., Ksouri R., et al. Potential assessment of Rumex vesicarius L. as a source of natural antioxidants and bioactive compounds. *Journal of Food Science and Technology*. 2014; 52:3549–3560.
45. Elzaawely A.A., Tawata S. Antioxidant Capacity and Phenolic Content of Rumex dentatus L . Grown in Egypt. *Journal of Crop Science and Biotechnology*. 2012; 15:59–64.
46. Chien S., Wu Y., Chen Z., et al. Naturally Occurring Anthraquinones : Chemistry and Therapeutic Potential in Autoimmune Diabetes. *Evidence-Based Complementary and Alternative Medicine*. 2015; 2015:1–13.
47. Khanal P., Jagdish B.M.P., Yasmin C. Anthraquinone Derivatives as an Immune Booster and their Therapeutic Option Against COVID - 19. *Natural Products and Bioprospecting*. 2020; 10:325–335.
48. Wegiera M.A., Smolarz H.D., Wianowska D., et al. Anthracene Derivatives in Some Species Of Rumex L. Genus. *Acta Societatis Botanicorum Poloniae*. 2007; 76:103–108.
49. Seitimova G.A., Shokan A.K., Tolstikova T.G., et al. Antiulcer Activity of Anthraquinone–Flavonoid Complex of Rumex tianschanicus Losinsk. *Molecules.* 2023; 28:2347.
50. Litvinenko Y.A., Muzychkina R.A. Phytochemical investigation of biologically active substances in certain Kazakhstan Rumex species. 1. *Chemistry of Natural Compounds.* 2003; 39:446–449.
51. Elazzouzi H., Zekri N. Total phenolic and flflavonoid contents of Anacyclus pyrethrum Link plant extracts and their Antioxidant activity. *Karbala International Journal of Modern Science.* 2019; 5:278–287.
52. Alkhatib R. Chemical Composition of Essential Oils Total Phenols and Antioxidant Activity of Achillea fragrantissima and A . santolina Grown in Syria. *Jordan Journal of Pharmaceutical Sciences*. 2024; 17: 594–602.
53. Kasote D.M., Katyare S.S., Hegde M.V., et al. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences.* 2015; 11:982–991.
54. Dulo B., Phan K., Githaiga J., et al. Natural Quinone Dyes: A Review on Structure, Extraction Techniques, Analysis and Application Potential. *Waste and Biomass Valorization.* 2021; 12: 6339–6374.
55. Zhang R, Huang C., Wu F., et al. Review on melanosis coli and anthraquinone-containing traditional Chinese herbs that cause melanosis coli. *Frontiers in Pharmacology*. 2023; 14:1–17

نباتُ الحمّاض التّفِه المنتشر برّياً في سوريا: تحليلٌ كيميائيٌّ نباتيّ وتقييمُ الفعاليّة المضادَّة للأكسدة في الزُّجاج لخلاصاتِ الأجزاءِ الهوائيّة والجذامير

***رند الخوري*[1] و رشا الخطيب[1]**

[1] قسم العقاقير والنباتات الطبية، كلية الصيدلة، جامعة دمشق، دمشق، الجمهورية العربية السورية.

ملخص

استُخدِمَ نبات الحمّاض التَّفِه *Rumex conglomeratus* Murr. (من الفصيلة البطباطية Polygonaceae) على نطاقٍ واسع شعبيّاً لعلاجِ العديد من الأمراض مثل الأمراض الجلديّة، الالتهابات، الإمساك، والسّرطان. وتعود الاستخدامات الشعبيّة والفعاليات الحيوية بشكلٍ رئيسيّ لغنى نباتاتِ جنس الحمّاض بالمستقلباتِ الثّانويّة الفعّالةِ حيوياً. تمثّل هذه الدِّراسة التقرير الأوّل للتّركيبِ الكيميائيّ والفعاليّة المضادّة للأكسدة للخلاصات المحضّرة من الأجزاء الهوائيّة والجذامير لنبات *Rumex conglomeratus*.. تمَّ في هذه الدّراسة تحضير الخلاصات المائيّة والإيثانوليّة، وأُجريت اختبارات الكشف الكيميائيّ الأوليّة، بالإضافة إلى تحديدِ إجمالي المحتوى من الفينولات، الفلافونوئيدات والأنتراكينونات، وتقييمِ الفعاليّة المضادّة للأكسدة باستخدام طرائق لونية يقاس فيها الامتصاص بجهاز UV-VIS spectrophotometer. أظهرت النّتائج أنّ *R. conglomeratus* مصدرٌ غنيٌّ بالمستقلباتِ الثّانويّة. وامتلكت خلاصةُ الجذامير الإيثانوليّة أعلى محتوى من الفينولات (502.55 ± 1.36 mg GAE / g DE) والأنتراكينونات (6.71 ± 0.106 mg RhE/g DE) كما امتلكت أعلى فعاليّة كاسحة لجذور DPPH الحرّة (IC50 = 5.40 ± 0.380 mg/L)، وقدرةً إرجاعيّة في اختبارِ FRAP (0.230 ± 0.004 at 200 mg/L) واختبار القدرة الكلّيّة المضادّة للأكسدة (321.41 ± 6.94 mg AAE / g DE). وكانت الفعالية المضادّة للأكسدة مرتبطة بشكل قوي بكلٍّ من المحتوى الفينولي والأنتراكينوني. تشير هذه النّتائج إلى إمكانيّة استخدام خلاصات نبات الحمّاض التَّفِه كمضادّ أكسدةٍ قويّ، أو حتى كعاملٍ ملّيّن. ومع ذلك من الضّروريّ إجراء المزيد من الأبحاث المستقبليّة لتحرّي المأمونيّة والفعاليّة، مع التّأكيدِ على مواصلةِ الاستكشاف لعزل وتحديد المركّبات الكيميائيّةِ الفعّالة حيوياً باستخدام طرائق الكروماتوغرافيا المختلفة، وتقييم العديد من الفعاليّات الحيويّة المُحتملة لخلاصات هذا النّبات الواعد.

الكلمات الدالة: الحماض التفه .Murr؛ الفصيلة البطباطية؛ الفينولات؛ الأنتراكينونات؛ الفلافونوئيدات؛ الفعالية المضادة للأكسدة.

---

* ***المؤلف المراسل: رند الخوري***

rand1.alkhoury@damascusuniversity.edu.sy

تاريخ استلام البحث 2024/02/29 وتاريخ قبوله للنشر 2024/05/18.

Pharmacists' Knowledge about Chronic Kidney Disease and its Management: Exploring Gaps and the Associated Factors

Anan S. Jarab[1,2], Maher Khdour[3], Tareq Mukattash[2], Haya subeh[3], Aseel Ghayada[3], Aseel Mhanna[3], Rania Ghanem*[3]

[1] College of Pharmacy, Al Ain University, Abu Dhabi, United Arab Emirates.
[2] Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Jordan.
[3] Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Al-Quds University, Jerusalem, Palestine.

ABSTRACT

Chronic kidney disease (CKD) is a worldwide public health problem. This study assessed pharmacists' knowledge about CKD and its therapeutic management. Online questionnaire was composed of three sections: sociodemographic factors, disease knowledge questions (range 0-11) and therapeutic knowledge-based questions (range 0-12). Disease and therapeutic knowledge indices were developed by calculating the median of the right answers for each part. Regression analysis was conducted to explore variables associated with CKD knowledge and its management. 352 pharmacists participated in the study. The median age was 31 (23–35) year, The majority of pharmacists (58%) were female, had bachelor's degree of pharmacy (84.7%), and had 1-3 years of experience (52.6%). The findings showed the participants exhibiting insufficient degree of knowledge about CKD knowledge with a median score of 7 (3-10) and a slightly higher degree of understanding in therapeutic knowledge compared to disease knowledge with median score 8 (4-11). Nevertheless, 79.5% of the sample members indicated that CKD is preventable. Increased age was associated with decreased knowledge (β = -0.038; P = 0.028). Hospital pharmacist increased the odds of having better knowledge about CKD (β = 0.044; P = 0.02). Higher academic achievement (β= 0.065; P=0.001) and being hospital pharmacist (β= 0.033; P= 0.02) were associated with improved CKD therapeutics'. The CKD knowledge among pharmacists is insufficient. Pharmacists need to be updated on CKD and encouraged to participate in formal training programs about renal disease management.

Keywords: Chronic kidney disease (CKD), Knowledge, Hospital Pharmacists, Community Pharmacists.

INTRODUCTION

Reduced kidney function, indicated by less than 60 mL/min/1.73 m2 of glomerular filtration rate (GFR), renal damage indicators, or both, for a minimum of three months, is known as chronic kidney disease (CKD) [1]. CKD is major public health concern [2] that most commonly develop in the elderly and people with concomitant conditions, particularly diabetes and hypertension.

Renal illness ranked as the 12th most common cause of death globally, in the study by Neuen et al., accounting for over 1.1 million fatalities [3]. According to the Ministry of Health in Palestine, renal failure accounts for 3.2% of all recorded fatalities in Palestine, making it the ninth leading cause of death [4]. In 2019, there were 11 dialysis units in the West Bank, of which 10 are owned by the Ministry of Health and include 240 devices for the Industrial College. Additionally, there are 5 units in the Gaza Strip that contain 102 sets for the Industrial College and one at An-Najah National University Hospital in

*Corresponding author: Rania Ghanem
rghanem@staff.alquds.edu
Received: 07/03/2024 Accepted: 18/05/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2463

 © 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

Nablus that contains 45 machines for the Industrial College. In 2020, there were 1573 patients who regularly received dialysis services in hospitals in the West Bank [4].

An average of 12 to 19 prescription drugs were given to patients with CKD; however, over 30% of patients did not take their medications as directed, which had a negative impact on treatment results [5]. With their expertise in identifying potential drug interactions, following the lab results, adjusting the dose , establishing monitoring strategies, avoiding medications that toxic to liver , managing adverse effects from kidney disease, patient education, and creating efficient medication therapy management, pharmacists perform an essential part as members of a kidney patient's healthcare team of CKD management [6]. Several studies demonstrate how well pharmacists can manage end-stage renal illness and chronic kidney disease (CKD), improving patient outcomes and patient care [7-9] .

The aim of this study was to bridge this gap in the literature by assessing professional pharmacists' knowledge of chronic dialysis, management, consequences, risk factors, and kidney disease detection.

METHODS

Study design and procedure

In this cross-sectional study, a self-reported online questionnaire was distributed on pharmacists in the community and hospital settings across different provenances in the West Bank via social media platforms (WhatsApp groups, Facebook, Twitter, etc.) in the period from March 1st, 2023 to May 30th, 2023 using convenience sampling technique.

Inclusion criteria

All pharmacists were included in the study if they had a Bachelor of Pharmacy (B. Pharm) degree or higher and had at least one year of professional experience and signed the consent form.

Exclusion criteria

Any pharmacist who cannot understand English survey or who did not sign the consent form.

Ethical approval

The Al-Quds University Ethical Approval Committee granted ethical approval for this investigation (Ref No: 284/REC/2023). The online completely anonymous questionnaire ensured the participants' confidentiality. No data revealing the participants' personal information was collected by the survey.

Sample size

The minimum sample size was determined by employing Epi Info and a sample size formula [10] This calculation was predicated on 4000 community pharmacists work in Palestine, with a 95% confidence level and a 5% precision level. Consequently, 352 was the bare minimum sample size necessary for this survey.

Study instrument

After a review of the literature the 26-item survey was adopted from [11]. The survey included 11-item disease knowledge questionnaire and 15-item therapeutic management questionnaire. The disease knowledge part evaluated knowledge in terms of definition, guidelines, symptoms, risk factors, complications and treatment perceptions. The therapeutic management part evaluated pharmacists' knowledge of key concepts of pharmacological and non-pharmacological management of CKD. The Cronbach alpha value of 0.741 demonstrated the reliability of the study survey. A panel of experts in the field including two nephrologists and three pharmacists evaluated the survey validity. To evaluate the relevance and clarity of the questionnaire items, ten community pharmacists participated in a pilot study of the survey, which was not included in the final data analysis

Statistical analysis

The data was reviewed for completeness and consistency before being analyzed with IBM SPSS Statistics. The demographic characteristics were

summarized using descriptive statistical analysis. For all variables, including awareness of chronic kidney disease, frequencies and percentages will be determined. This comprises general understanding, prevention, treatment strategies, and prescribing. To test for differences in response, Fisher's exact test will be performed. Continuous data were analyzed using the Student's t-test and ANOVA tests, and were reported as means and standard deviations (M±SD). To test for differences in response, Fisher's exact test will be performed. On both illness and treatment knowledge, bivariate and multiple regressions were used and a *p*. value set of <0.05 was considered significant.

RESULTS

Most of the study participants were female (58%), aged between 23-35 years (65.6%), held a Bachelor degree in pharmacy (84.7%), had professional experience of less than 3 years (52.6%), and were employed in a community pharmacy setting (65.9%). Further details are presented in Table (1). As shown in figure (1), 48% of the participants reported poor disease knowledge and 60% reported poor knowledge about CKD management.

The result of this study suggests that there is a need for increased pharmacist understanding of certain elements of chronic kidney disease (CKD). The detection of early signs is a key area where information is limited, with just 55% identifying decreasing urine output as a symptom. There is also a misunderstanding of specific diagnostic criteria, specifically the classification of CKD based on albuminuria levels, which is acknowledged by just 48% of respondents. Furthermore, there are significant misconceptions about the treatability of CKD, with a low percentage correctly knowing that disease is incurable with surgery or medicine. Furthermore, there is a lack of knowledge regarding specific risk factors and consequences, such as dyslipidemia (31%), and metabolic bone disease (29.8%), as shown in Table (2).

The study highlights the need for more understanding in a few areas related to the management of chronic kidney disease (CKD). The use of diuretics in acute kidney injury (AKI), which is only acknowledged by 25.7% of respondents, is one area with significant knowledge gaps. Furthermore, fewer than half (46%) knew exactly what the recommendations were for loading doses in patients with CKD. It is also necessary to raise awareness of dietary guidelines and specific pharmaceutical uses in cases of AKI and CKD. For example, few people were aware of the usage of metformin and ACE inhibitors at particular phases of chronic kidney disease. Results of univariate analysis showed a statistically difference in the disease knowledge score was in those group of pharmacists depending on age , number of years of experience , years of experience as a hospital pharmacist and academic achievement as a PhD graduate p value = 0.001 , 0.041, 0.001 and 0.037 respectively (Table 4). Statistically difference in the medication knowledge score was in those group of pharmacists depending on age and years of experience as a hospital pharmacists P value = 0.038, 0.031 respectively. Table 3 provides a summary of the percentage of accurate answers to each question on the CKD treatment questionnaire.

The multivariate analysis revealed that for every 1-year increase in the age of the pharmacist, the disease knowledge score decreased by the 0.031. (β = -0.031; p value =0.028) (Table 2). On the other hand, there was a positive association between the disease knowledge score and being a hospital pharmacist. (β = 0.044; p value =0.001). Results also showed that being a hospital pharmacist (β = 0.033; p value =0.028) and increased years of experience (β = 0.065; p value =0.001) increased the odds of having improved knowledge about the therapeutic management of CKD.

Table 1: Demographic characteristics of the study participants.

Variable	Number of patients (N=352)	% of Patients
Gender		
Male	148	42.0%
Female	204	58.0%
Age		
23-35 years	231	65.6%
36-45 years	92	26.1%
> 45	29	8.3%
Governorate		
Jerusalem	63	17.9%
Ramallah	72	20.5%
Bethlehem	80	22.7%
Hebron	40	11.4%
Nablus	32	9.1%
Jenin	16	4.5%
Jericho	10	2.8%
Tulkarm	15	4.3%
Tubas	4	1.1%
Salfit	5	1.4%
Qalqilya	6	1.7%
Gaza	9	2.6%
Jerusalem	63	17.9%
Academic achievement		
Bachelor's degree	298	84.7%
Master's degree	40	11.4%
Ph.D.	14	4.0%
Place of residence		
City	230	65.3%
Village	100	28.4%
Camp	22	6.3%
Employer		
Community Pharmacy	232	65.9%
Hospital Pharmacy	120	34.1%
Years of experience		
1-3 years	185	52.6%
4-7 years	68	19.3%
8-10 years	69	19.6%
>10 years	30	8.5%

Table 2: Percentage of Correct Response to Individual Items on the CKD knowledge Questionnaire.

Disease Knowledge Items	Response N (%)	
	Correct	**wrong**
CKD is changes in kidney structure or function that have persisted for more than three months and have an impact on health	156 (91.2)	15 (8.8)
What is/are the symptom of early kidney disease (Tick as applicable)		
Back Pain	103(60)	68(40)
Reduce in urine output	95(55)	76(45)
Weight/ appetite loss	111(65)	60(35)
Tiredness	132(77)	39(23)
According to the KDOQI recommendations, CKD is divided into 5 classes (G1 through G5) based on GFR levels	131 (76.6)	40 (23.4)
According to the KDOQI criteria, there are three groups (A1 to A3) for CKD based on albuminuria values	89 (52.0)	82 (48.0)
Is increased serum creatinine alone a poorer indicator of a deterioration in renal function than eGFR?	106 (62.0)	65 (38.0)
Is low eGFR associated with normal serum creatinine, normal urine analysis, and normal USG possible with an age-related decrease in eGFR	114 (66.7)	57 (33.3)
When estimating GFR, the Cockroft-Gault equation performs better than the MDRD equation.	47 (27.5)	124 (72.5)
The risk factors listed below should be taken into account while estimating the prognosis for CKD (Tick as applicable)		
Elevated blood pressure	111 (64.9)	60 (35.1)
Hyperglycemia	97 (56.7)	74 (43.3)
Dyslipidemia	53 (31.0)	118 (69.0)
History of cardiovascular disease	88 (51.5)	83 (48.5)
Chronic use of NSAIDs, lithium, cyclosporine	101 (59.1)	70 (40.9)
Glomerulonephritis	114 (66.7)	57 (33.3)
The 2017 ACC/AHA guidelines state that patients with CKD should aim for a blood pressure of less than 130/80 mmHg; those whose blood pressure is higher than 130 mmHg will be considered hypertensive.	131 (76.6)	40 (23.4)
Every patient with chronic kidney disease (CKD) should have ongoing monitoring for the following consequences. (Tick as applicable)		
Anemia	77 (45.0)	94 (55.0)
Metabolic bone disease	51 (29.8)	120 (70.2)
Hyperkalemia	118 (69.0)	53 (31.0)
Acidosis	85 (49.7)	86 (50.3)
Edema	106 (62.0)	65 (38.0)
Acute Kidney Injury	108 (63.2)	63 (36.8)
Every CKD patient has to be aware that they have a significant chance of suffering an acute kidney injury (AKI).	134 (78.4)	37 (21.6)
Regarding CKD		
Kidney disease can be prevented	135 (79.4)	36 (20.6)
Kidney disease can be cured by medication	17(9.7)	154(90.3)
Kidney disease can be cured by surgery	19(10.9)	152(89.1)
Overall correct answer (median)	8 (3-11)	

Table 3: Percentage of Correct Response to Individual Items on the CKD therapeutic knowledge Questionnaire

Therapy management Knowledge Items	Response N (%)	
	Correct	**Wrong**
Are ACE inhibitors the first-choice medications for treating both diabetic and non-diabetic patients' CKD?	91(53)	80(47)
Every patient with chronic kidney disease who is at risk of acute kidney injury should follow a high-protein diet (AKI)	112 (65.5)	59 (34.5)
Guidelines advise CKD patients with AKI to maintain their level of hydration by using isotonic crystalloid fluids.	89 (52.0)	82 (48.0)
When electrolyte and fluid levels in CKD patients with AKI suddenly shift, dialysis should be started.	106 (62.0)	65 (38.0)
Diuretics are advised for CKD patients with AKI in order to enhance renal function.	44 (25.7)	127 (74.3)
Patients with AKI receiving dialysis who are not at risk of bleeding are advised to receive anticoagulation medication with enoxaparin or unfractionated heparin	107 (62.6)	64 (37.4)
Since valproic acid, an anticonvulsant medication, can be dialyzed, does it need to be taken again after dialysis?	82(47.9)	89(52.1)
Iron treatment is advised for anemic CKD patients.	111 (64.9)	60 (35.1)
At Hb > 10 g/dl, erythropoietin therapy is not advised	111 (64.9)	60 (35.1)
Patients with CKD who have anemia and a systemic infection should continue receiving IV iron dextran.	41 (24.0)	130 (76.0)
Patients with CKD who are at risk of mineral and bone disorders should receive phosphate lowering therapy with phosphate binders.	107 (62.6)	64 (37.4)
Patients with CKD do not require modifications to loading doses.	79(46)	92(54)
If there is a more than 30% increase in serum creatinine, ACE inhibitors should be stopped.	120(70)	51(30)
Patients with stage 5 CKD and a GFR of less than 15 ml/min can take metformin.	96(56)	75(44)
When treating G3a-G5 stage CKD patients, should the dosage of calcium-based phosphate binders be avoided?	109(63.7)	62(36.3)
Overall correct answer (median)	10 (4-12)	

Table 4: Univariate analysis of various factors affecting Pharmacist's knowledge

		Disease Knowledge		**P. Value**
		Poor n (%)	**Good n (%)**	
Gender	Male	85(49.3)	86(50.7)	0.11
	Female	95(55.4)	76(44.6)	
Age	23-35 years	100(58.8)	71(41.2)	
	36-45 years	46(26.8)	125(73.2)	0.001**
	> 45	47(27.3)	124(72.7)	
Experience	1-3 years	69(40.1)	102(58.9)	0.041*
	4-7 years	105(61.4)	66(38.6)	
	8-10	63(37.1)	108(62.9)	
	> 10			
Employer	Community Pharmacy	103(60.1)	68(39.9)	0.001**
	Hospital Pharmacy	47(27.4)	124(72.6)	
Academy achievement	Bachelor's degree	96(56.0)	75(44.0)	
	Master's degree	60(35.0)	111(65.8)	
	Ph.D.	69(40.1)	102(58.9)	0.037*
Place of residency	City	93(54.1)	78(55.9)	0.08
	Village	108(63.4)	63(41.6)	
	Camp	104(61.0)	67(39.0)	
		Medication Knowledge		P. Value
		Poor n (%)	Good n (%)	
Gender	Male	104(61.0)	67 (39.0)	0.21*
	Female	96(56.1)	75(43.9)	
Age	23-35 years	101(58.8)	70(41.2)	
	36-45 years	61(35.8)	110(64.2)	0.038*
	> 45	69 (40.4)	102(59.6)	
Experience	1-3 years	69(40.1)	102(58.9)	0.031*
	4-7 years	105(61.4)	66(38.6)	
	8-10	63(37.1)	108(62.9)	
	> 10			
Employer	Community Pharmacy	82(48.2)	89(51.8)	0.001**
	Hospital Pharmacy	39(22.9)	132 (77.1)	
Academy achievement	Bachelor's degree	103(60.0)	68(40.0)	
	Master's degree	38(22.2)	133(77.8)	
	Ph.D.	69(40.1)	102(58.9)	0.001**
Place of residency	City	53(31.1)	118(69.9)	0.02*
	Village	103(60.2)	68(39.8)	
	Camp	101(58.9)	70(40.1)	

Table 5: Multiple regression analysis for CKD disease and therapeutics knowledge

	Disease Knowledge				Therapeutic Knowledge			
Variable	***β***	**SE**	**t-statistics**	**P. Value**	***β***	**SE**	**t-statistics**	**P. Value**
Constant	0.191	1.393	0.139	0.893	0.191	1.393	0.139	0.893
Gender	0.229	0.587	0.389	0.711	-.007	0.04	-0.177	0.711
Years of practice	0.483	0.297	1.628	0.169	-0.201	0.463	-0.433	0.675
Age	-0.031	0.311	-2.615	0.028	0.017	0.032	0.546	0.586
Hospital pharmacy	0.044	0.014	3.142	0.020	0.033	0.010	3.142	0.028
Education degree [c]	0.333	0.293	1.13	0.124	0.065	0.017	3.89	0.001
Residency[d]	0.739	0.390	1.912	0.071	0.031	0.016	1.93	0.066
Pharmacy owner [e]	-0.201	0.463	-0.433	0.675	-	-	-	-

N=352. Adjusted R^2 =0.684. [a]Gender coded, 1= male, 0 =female. [b]Years of practice coded, 1= <3 years, 2= 4-10, 3= 11-20, 4= >20. [c]Education 1= PhD, Master's degree, 0= Bachelor. [d]Residency coded,1 =middle, 2 = north, 3 = south. Pharmacy owner coded, 1 = yes, 0 = no.

DISCUSSION

In addition to be the first study which evaluated pharmacists' knowledge about CKD and its therapeutic management in Palestine, earlier research studies reported inconsistent findings of knowledge levels and its associated factors, which necessitates further investigations for this purpose.

The respondents in this study have modest understanding about CKD, with a median of seven correct responses out of eleven. An earlier study reported that pharmacists were equally distributed to have good or poor knowledge with a mean knowledge score of 54.76 ± 16.3 percent [11-13].

The high recognition percentage (91.2%) of the CKD definition in this survey is encouraging, indicating that respondents had a sound basic understanding. However, the disparity in the detection of symptoms such as fatigue (77%) and decreased urine output (55%) suggests a need for increased education on early CKD indications. Most of the present study participants were able to classify CKD using GFR values, demonstrating knowledge of the KDOQI recommendations. On the other hand, the lesser understanding of albuminuria-based classification (48%) indicates a specific area for educational improvement.

The disparity in identifying CKD risk factors in this study might have an impact on preventive actions. Similarly, the disparity in the detection of consequences such as hyperkalemia and metabolic bone disease highlights the need for comprehensive education on CKD outcomes. The low percentage of respondents who recognized the incurability of CKD through surgery and medicine is a major concern, since it may have an impact on patient counseling and care, which underlined the need for increased education on CKD management.

The fact that 53% of people are aware that ACE inhibitors are the first-line treatment for CKD, which is lower than the findings of another study [14], highlights a need for more education as ACE inhibitors represent a cornerstone in CKD management. On the other hand, a higher proportion of the current study participants were able to identify erythropoietin and iron therapy use guidelines for anemia management. Understanding of the necessity of dialysis, as well as the appropriate use of anticoagulant medicine in AKI patients on dialyses, is rather high, demonstrating superior awareness in these specific therapy elements. However, the majority of the participants were unable to recognize diuretics function in AKI. A more encouraging response about the use of dietary advice and the administration of isotonic crystalloid fluids for AKI management was reported in this study.

Regression analysis showed that knowledge was age

dependent, with pharmacists of lower ages having the best Disease knowledge levels. These results was in agreement with a study conducted on healthcare professionals in Saudi Arabia, which discovered that being under 40 years old is related with higher knowledge scores [15]. The outcomes of disease knowledge among pharmacists of various levels of education were startlingly divergent, which may be cause for concern. It was discovered that pharmacists with higher Postgraduate degrees, such as Master's and PhD degrees, have stronger clinical understanding than those with only a Bachelor's degree. One key explanation for that advanced degrees foster critical thinking and problem-solving skills, which are vital in clinical practice. Pharmacists with postgraduate degrees are better equipped to assess complex patient cases, interpret medical literature, and make informed decisions, all of which contribute to superior clinical understanding [16].

Consistent with the findings of Teh's team [17], being a hospital pharmacist and increased years of experience were associated with increased pharmacists' therapeutic knowledge about CKD. Hospital pharmacists are regularly engaged in medication evaluations and dose modifications that would enrich disease management information. Furthermore, was associated with improved therapeutic knowledge, which is consistent with earlier research finding [18-21].

One notable advantage of this study is its pioneering nature; it is the first to test pharmacists' knowledge of chronic renal disease and drugs used in Palestine. The study findings can be used in a variety of practical ways to improve pharmacists' knowledge of CKD and improve patient health outcomes. For starters, introducing CKD-specific continuing education programs can keep pharmacists up to date on the most recent treatment guidelines and management practices. Second, holding interactive seminars and case study discussions might help them better comprehend the complexity of CKD. Third, by incorporating CKD management into regular pharmacy practice, such as through medication review guidelines for CKD patients, pharmacists can ensure they are actively involved in patient care. Creating easily accessible reference materials and decision support tools suited for pharmacy practice can also help with quick and accurate information retrieval during patient consultations. These measures can help pharmacists become more knowledgeable, resulting in better medication management and health outcomes for CKD patients. However, one major restriction is that online questionnaires do not provide the human engagement that in-person or phone interviews do. As a result, data quality may suffer since respondents may not request clarification on confusing questions.

CONCLUSION

There is an insufficient of understanding about CKD treatment. Hospital pharmacists are better knowledgeable about CKD treatment. There is a need to refresh pharmacists' renal disease knowledge and urge them to participate in structured kidney disease management training programs. Professional clinical experience is as crucial as the pharmacist's level of education. To generate pharmacists with stronger clinical abilities, the pharmacy teaching and training system should be upgraded.

AUTHOR'S CONTRIBUTION: Study concept and design: R.G., and M. K; analysis and interpretation of data: R.G., M.K. and A.J.; drafting of the manuscript: A.J. and R.G.; critical revision of the manuscript for important intellectual content: R.G., T.M., and M.K.; statistical analysis: R.G., M.K., A.J., T.M., H.S, A.G., and A.M. Data collection: H.S., A.G., and A.M. All authors contributed to the drafting and critical review of the manuscript and have approved the final draft of the manuscript.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY

The dataset presented in the study is available on request from the corresponding author during submission or after its publication.

ACKNOWLEGMENT

The authors would like to thank all the participants for providing the data used in this study.

REFERENCES

1. Levin A., Stevens P.E., Bilous R.W., Coresh J., De Francisco A.L., De Jong P.E., Griffith K.E., Hemmelgarn B.R., Iseki K., Lamb E.J. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney international supplements.* 2013; 3(1):1-150.
2. Imtiaz S., Salman B., Qureshi R., Drohlia M.F. and Ahmad A. A review of the epidemiology of chronic kidney disease in Pakistan: A global and regional perspective. *Saudi Journal of Kidney Diseases and Transplantation.* 2018; 29(6):1441-1451.
3. Neuen B.L., Chadban S.J., Demaio A.R., Johnson D.W. and Perkovic V. Chronic kidney disease and the global NCDs agenda. In., vol. 2: *BMJ Specialist Journals*; 2017: e000380.
4. MOH: Health Annual Report, Palestine 2020. 2021.
5. Manan W.Z.W., Wei F.C., Abd Rahman F. and Ming L.C. Pharmaceutical care to improve medication knowledge among patient with chronic kidney disease. *Journal of Pharmacy & Bioallied Sciences.* 2016, 8(3):263.
6. Schütze A., Hohmann C., Haubitz M., Radziwill R. and Benöhr P. Medicines optimization for patients with chronic kidney disease in the outpatient setting: the role of the clinical pharmacist. *International Journal of Pharmacy Practice.* 2021; 29(6):587-597.
7. Inoue Y., Takikawa M., Morita Y., Takao K., Kanamoto I. and Sugibayashi K. A comparison of pharmacists' role functions across various nations: the importance of screening. *Research in Social and Administrative Pharmacy.* 2016; 12(2):347-354.
8. Gheewala P.A., Peterson G.M., Curtain C.M., Nishtala P.S., Hannan P.J. and Castelino R.L. Impact of the pharmacist medication review services on drug-related problems and potentially inappropriate prescribing of renally cleared medications in residents of aged care facilities. *Drugs & Aging.* 2014; 31:825-835.
9. Esmaeili M., Sarmadian R., Fatahibayat G., Yousefichaijan P. and Habibi D. Evaluation of Blood Pressure in Children Treated with Ceftriaxone: A Case-Control Study. *Jordan Journal of Pharmaceutical Sciences.* 2023; 16(3):508-516.
10. Daniel W. Biostatistics: A Foundation for analysis in the health sciences, 7th edR Wiley. New York. 1999; 141(2).
11. Syed Sulaiman S.A. and Tariq M.H. Evaluation of pharmacist's knowledge regarding chronic kidney disease. *Archives of Pharmacy Practice.* 2020; 11(4).
12. Khdour M.R., Kurdi M., Hallak H.O., Jarab A.S., Dweib M. and Al-Shahed Q.N. Pharmacistsâ€™ Knowledge, Attitudes and Practices Towards Herbal Remedies In West Bank, Palestine. *International Archives of Medicine.* 2016; 9.
13. Khdour M., Kurdi M., Hallak H., Dweib M. and Al-Shahed Q. Pharmacists' knowledge, attitudes, and practices towards herbal remedies in the West Bank: a cross-sectional study. *The Lancet,* 2018; 391:S17.

14. Zhang Y., He D., Zhang W., Xing Y., Guo Y., Wang F., Jia J., Yan T., Liu Y. and Lin S. ACE inhibitor benefit to kidney and cardiovascular outcomes for patients with non-dialysis chronic kidney disease stages 3–5: a network meta-analysis of randomised clinical trials. *Drugs*. 2020; 80:797-811.
15. Alrabiah A.M., Elsaid T. and Tourkmani A. Determinants of family medicine physicians' knowledge and application of asthma management guidelines at primary healthcare centers in Riyadh, Saudi Arabia. *Journal of Family Medicine and Primary Care*. 2018; 7(5):927.
16. Persky A.M., Medina M.S. and Castleberry A.N. Developing critical thinking skills in pharmacy students. *American Journal of Pharmaceutical Education*. 2019; 83(2).
17. Teh X.R. and Lee S.W. Pharmacists' attitude, self-reported knowledge and practice of dosage adjustment among chronic kidney disease patients in Malaysia. *Journal of Pharmacy Practice and Research*. 2019; 49(2):179-185.
18. Valdez C., Namdar R. and Valuck R. Impact of pharmacy experience, GPA, age, education, and therapeutic review on knowledge retention and clinical confidence. *Currents in Pharmacy Teaching and Learning*. 2013; 5(5):358-364.
19. Ghanem R.E., Mostafa S.H., Abu Hamamda N.K. and Khdour M.R. Knowledge, attitudes, and practices of community pharmacists toward the management of acne vulgaris in Palestine: a cross-sectional study. *International Journal of Dermatology*. 2020; 59(4):506-512.
20. Masadeh M.M., Harun S.N. and Alrabadi N. Evaluating the Validity and Reliability of Questionnaires Measuring Knowledge, Attitudes, and Practices Towards Antibiotic Resistance Among Youths: A Systematic Review Protocol. *Jordan Journal of Pharmaceutical Sciences*. 2024; 17(2):362-370.
21. Hjazeen R. Community Pharmacists' Perspectives toward Continuing Professional Development: A Qualitative Study. *Jordan Journal of Pharmaceutical Sciences*. 2023; 16(2):449-449.

معرفة الصيادلة بمرض الكلى المزمن وإدارته: استكشاف الثغرات و العوامل المرتبطة بها

عنان جراب[1،2]، ماهر خضور[3]، طارق مقطش[2]، هيا صبح[3]، اسيل غياظة[3]، اسيل مهنا[3]، رانيا غانم[3]*

[1] كلية الصيدلة، جامعة العين، أبو ظبي، الإمارات العربية المتحدة.

[2] قسم الصيدلة السريرية، كلية الصيدلة، جامعة العلوم والتكنولوجيا، إربد، اللأردن.

[3] قسم الأدوية والعلاجات، كلية الصيدلة، جامعة القدس، القدس، فلسطين.

ملخص

مرض الكلى المزمن (CKD) هو مشكلة صحية عامة في جميع أنحاء العالم. قيمت هذه الدراسة معرفة الصيادلة حول CKD وإدارة علاجه. تكون الاستبيان المرسل عبر الإنترنت من ثلاثة أقسام: العوامل الاجتماعية والديموغرافية، أسئلة معرفة المرض (العلامات 0−11) والأسئلة العلاجية القائمة على المعرفة (العلامات 0−12). وقد تم تطوير مؤشرات المرض والمعرفة العلاجية من خلال حساب متوسط الإجابات الصحيحة لكل جزء. تم إجراء تحليل الانحدار لاستكشاف المتغيرات المرتبطة بمعرفة CKD وإدارتها. شارك في الدراسة 352 صيدلاني. أظهرت النتائج أن لدى المشاركين درجة متواضعة من المعرفة حول مرض الكلى المزمن مع متوسط درجة 7 (3−10) ودرجة أعلى قليلا من الفهم في المعرفة العلاجية مقارنة بمعرفتهم بالمرض مع متوسط درجة 8 (4−11). ومع ذلك، أشار 79.5٪ من أعضاء العينة إلى أن CKD يمكن الوقاية منه. في حين أن تقدم العمرلدى عينة الدراسة كان مرتبطا بانخفاض المعرفة (β = -0.038. ع = 0.028). أشارت الدراسة إلى أنه لدى صيادلة المستشفيات معرفة أفضل حول CKD (β = 0.044 ؛ ع = 0.02). كما أضحت الدراسة أن الصيادلة ذوي التحصيل الأكاديمي العالي (β = 0.065؛ P = 0.001) و من هم من صيادلة المستشفيات (β = 0.033 ؛ P = 0.02) كانت لديهم معرفة أفضل فيما يخص علاجات CKD. معرفة CKD بين الصيادلة كانت غير كافية، والصيادلة بحاجة إلى تحديث معلوماتهم حول مرض CKD و إدارة علاجه، وتشجيعهم على المشاركة في برامج التدريب الرسمية المتعلقة ذلك.

الكلمات الدالة: مرض الكلى المزمن (CKD)، معرفة، صيادلة المجتمع، صيادلة المستشفيات.

* ***المؤلف المراسل: رانيا غانم***

rghanem@staff.alquds.edu

تاريخ استلام البحث 2024/03/07 وتاريخ قبوله للنشر 2024/05/18.

Jordan Journal of Pharmaceutical Sciences, Volume 17, No. 4, 2024

Design, Synthesis, Molecular docking and Biological Evaluation of Novel Leucine Derived Sulfamoyl Pentanamides as Antimicrobial and Antioxidant Agents

Melford C. Egbujor*[1], Vivian, I. Okonkwo[2], Ugomma C. Onyeije*[3], Chigbundu N. Emeruwa[4], Ogbonna C. Nkuzinna[5], Pius I. Egwuatu[6], Ifeanyi S. Amasiatu[7], Alisa C. Onyemeziri[8], and Uchechukwu C. Okoro[9]

[1]Department of Chemistry, Federal University Otuoke, Bayelsa State, Nigeria
[2]Department of Science Laboratory Technology, University of Nigeria, Nsukka, Nigeria.
[3]Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria
[4]Department of Chemical Sciences, Rhema University Nigeria, Aba, Abia State, Nigeria
[5]Department of Chemical Engineering, Federal University of Technology, Owerri, Nigeria
[6]Department of Microbiology, Renaissance University, Ugbawka, Enugu State.
[7]Department of Biochemistry, Renaissance University, Ugbawka, Enugu State.
[8]Department of Chemistry, Federal University of Technology, Owerri, Nigeria.
[9]Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Nigeria

ABSTRACT

The preponderance of microbial and oxidative stress-mediated diseases is quite alarming. The need for novel drug development is highlighted by the fact that antimicrobial resistance is rising and many current antioxidant drugs only provide little symptomatic alleviation. The aim of this work was to synthesize leucine derived sulfamoyl pentanamides with antioxidant and antimicrobial activities. New leucine-based sulfamoyl pentanamides were synthesized and elemental analysis, 1H-NMR, 13C-NMR, and FTIR were used to elucidate their structures. They underwent molecular docking investigations as well as in vitro antioxidant and antimicrobial activity analyses. Compound 5a (0.60 gm/ml) was the most active compound against *Pseudomonas aeroginosa*, whereas compound 5f (0.30-0.40 mg/ml) was the most effective antibacterial agent against *E. Coli, S. typhi, S. aureus, and B. subtilis*. The compounds with the best antifungal activity against *C. albican* and *A. niger*, respectively, were 5g (0.80 mg/ml) and 5e (0.50 mg/ml). In the in vitro antioxidant assessment, compounds 5g (1.174µg/ml) and 5h (1.172µg/ml) exhibited similar antioxidant activity to ascorbic acid (IC50 1.001µglml). In addition, most of the target compounds have relatively strong antibacterial, antifungal, and antioxidant potentials, according to molecular docking study. Since every target compound complied with Lipinski's rule of five, it is likely that they might be used as therapeutic candidates to treat oxidative stress-related illnesses and microbial infections.

Keywords: pentanamides; leucine; sulfonamides; antimicrobial; antioxidant; synthesis.

INTRODUCTION

The global health sector is seriously threatened by the prevalence of oxidative stress-related diseases and organisms that are resistant to antibiotics. Among the biggest health problems of the twenty-first century are oxidative stress and microbial infections.[1,2] Thus, in order to elicit dual mechanisms of action and minimize drug resistance, this situation highlights the necessity for the development of drugs through hybrid pharmacophoric strategies that allow the linking of two or more bioactive moieties with distinct pharmacological activities into a drug molecule.[3-5] Combining certain amino acids with sulfonamides and

***Corresponding author: Melford C. Egbujor**
egbujormc@fuotuoke.edu.ng
Received: 08/03/2024 Accepted: 18/05/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2467

© 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

carboxamides has been shown to exhibit excellent inhibitory activities against diseases linked to oxidative stress and microbial infections.[6-8] According to Fox et al.'s [9] research, leucine is a non-polar aliphatic α-amino acid with excellent antimicrobial potential. It was shown to inhibit Lactobacillus arabinosus strains from growing. Similar to this, it was observed that leucine improved the antibiotic action of ramoplanin, a glycolipodepsipeptide obtained by the fermentation of Actaioplanes sp.[10] Like other branched-chain amino acids, leucine is also known to possess antioxidant properties. Jin et al.[11] found that leucine has an inhibitory effect on lipid peroxidation and nitric oxide scavenging activity, and they proposed that leucine could be used to produce antioxidant products for the food or pharmaceutical industries. Numerous studies have demonstrated the antimicrobial[12-15] and antioxidant[16,17] properties of sulfonamide moieties, among other properties. Additionally, it has been documented that sulfonamides not only neutralize free radicals but also trigger nuclear factor erythroid 2-related factor 2 (NRF2), the primary modulator of an organism's endogenous antioxidant responses.[18,19] Carboxamides also display a wide range of biological activity.[20] They have the potential to be used as medications to treat oxidative stress and microbial infections.[13] In view of improving drug potency through synergism, we proposed that integrating leucine, pentanamide, and sulfonamide moieties into a single compound by simple and effective synthesis would be crucial for increasing therapeutic efficacy through synergism. In view of combating health issues linked to oxidative stress and antimicrobial resistance, the goal of this work was to synthesize leucine-based sulfamoyl pentanamides with enhanced antimicrobial and antioxidant properties.

MATERIAL AND METHODS

Reagents and Instrumentation Sigma Aldrich provided the chemicals. The compounds' melting points were obtained using an electrothermal melting point equipment. 8400s Fourier Transform Infrared spectrometer was used to determine the FT-IR spectra of the compounds. In the Department of Chemistry at the Indian Institute of Technology, Kanpur, India, the 1H- and 13C-NMR analyses were conducted at 400MHz using DMSO. Chemical changes were represented in parts per million, with tetramethylsilane serving as the standard. The elemental analyzer (Exeter Analytical Inc. model: CE440) was used to perform the elemental analysis. For all processes that needed inert conditions, nitrogen gas was utilized.

Chemistry

Synthesis of 4-methyl-2-{[(4-methylphenyl)sulphonyl]amino}pentanoic acid. Water (15 ml) and L-leucine (12 mmol) were mixed in a 100 ml beaker. NaOH (26.30 mmol) was then added, and the mixture was swirled until the solutes were completely dissolved. The different sulphonyl chlorides (1a-b) (30 mmol) were added to the solution in five sections over the course of an hour after it had cooled to zero degrees Celsius. After four hours of stirring at room temperature, 2M hydrochloric acid was added to acidify the mixture to pH 2 in order to promote crystallization. TLC (MeOH/DCM, 1:9) was used to track the reaction process. The products were separated using suction filtration after it was let to settle for a full day. After drying and washing with tartaric acid (pH 2.2), the products yielded chemicals (3a-b) in their analytical grade excellent.

Acetylation of the Sulfamoyl Carboxylic Acids (3a-b). In order to guarantee homogeneity, a beaker was filled precisely with the 1 mmol of sulfamoyl carboxylic acids (3a–b), 25 ml of distilled water, and 9 ml of concentrated HCl. The mixture was then rapidly agitated. Then 150.29 mmol of NaCO3 was dissolved in 50 ml of distilled water in a different 100 ml beaker. The sulfamoyl carboxylic acid solution was filled with the sodium acetate solution and 13.5 ml of acetic anhydride was added in three increments over the course of an hour. To extract the N-

acetylated sulfamoyl carboxylic acids (4a-b) in excellent yields, the mixture was mixed, submerged in an ice bath for an hour, and then filtered and rinsed with water.

Chlorination and ammonolysis of 4a-b

Chlorination. When a three-necked flask with a magnetic bar was filled with sulfamoyl carboxylic acids (4a–b) (1 mmol) and acetone (10 ml), it was cooled to 0°C. Excess thionyl chloride was removed from the mixture by stirring it at 80°C under reflux for three hours before transferring it to an 80°C water bath. To achieve complete evaporation of thionyl chloride and produce acid chloride intermediate, 20 ml of acetone was added and the process was repeated twice.

Ammonolysis. After dissolving the aforementioned acid chloride in 20 ml of acetone, the mixture was chilled to zero degrees Celsius. Crystallization occured when 2 ml of ammonia and 1M NaOH were added, and the liquid was then left to settle for 24 hours before being filtered and cleaned with acetone to extract the remaining material.

2{Acetyl[(4-methylphenyl)sulfonyl]amino}-4-methylpentanamide (5a). Yield; 2.95g (90.1%), mp.215-216^{o}C, IR (KBr) cm^{-1}: 3370(N-H), 2061(C-H aliphatic), 2001(C-H aromatic), 1722,1690(C=O), 1694,1646 (C=C), 1326, 1151(2S=O), 1121(SO_2-NH), 1032(C-N), 682(Ar-H). ^{1}HNMR (DMSO, 400 MHz) δ: 7.32 (d, J = 7.5Hz, 2H, Ar), 6.60 (m, 2H, ArH), 5.69 (s, 2H, NH_2), 3.45 (s, 3H, CH_3-C=O), 2.46 (s, 3H, CH_3-Ar), 1.27 (s, 6H, 2CH_3-CH). ^{13}CNMR (DMSO, 400 MHz)δ: 170.11, 169.23(C=O), 137.08, 133.77, 133.59 132.46, 131.92, 129.21 (aromatic carbon), 67.85, 60.65, 53.68, 50.68, 40.02, 39.82, 39.63 (aliphatic carbons). Anal.calcd (%). for $C_{15}H_{22}N_2O_4S$ (326.41): C: 55.15, H: 6.79, N: 8.58, S: 9.80. Found: C: 55.18, H: 6.81, N: 8.60, S: 9.78.

2-[Acetyl(phenylsulfonyl)amino]-4-methylpentanamide (5b). Yield; 3.09g(92.3%), mp.135-136 ^{o}C, IR (KBr)cm^{-1}: 3257(N-H), 2958(C-H aliphatic), 1996(C-H aromatic), 1720, 1689 (C=O), 1640(C=C), 1344, 1307(2S=O), 1162(SO_2-NH), 1092(C-N), 752(Ar-H). ^{1}HNMR (C_6D_6/DMSO/$CDCl_3$, 400MHz)δ: 8.107 (s, 2H, ArH), 7.147 (s, 2H, ArH), 5.689 (s, 2H, NH_2), 2.209(s, 3H, CH_3-C=O). ^{13}CNMR(C_6D_6, 400 MHz)δ: 178.67, 172.42, 2(C=O),138.62, 138.43, 133.52, 132.97, 132.73, 132.49 (aromatic carbons), 84.09, 83.76, 83.42, 44.99, 44.79, 44.58. ^{13}CNMR(C_6D_6, 400 MHz)δ: 171.67, 170.42, 2(C=O), 138.62, 138.43, 133.52, 132.97, 132.73, 132.50 (aromatic carbons), 84.09, 83.76, 83.42, 44.99, 44.79, 44.58. Anal.calcd.(%) for $C_{14}H_{20}N_2O_4S$ (312.38): C: 53.78, H: 6.40, N:8.96, S: 10.24. Found: C: 53.80, H: 6.38, N: 8.99, S: 10.21

Leucine-based Sulfamoyl Pentanamide Synthesis via Nickel-Catalyzed Reaction:

Bis(triphenylphosphine)nickel(ii)chloride Preparation. Using the Venanzi[21] reaction protocol, the coordination compound was made by dissolving 10 mmol of nickel (II) chloride hexahydrate in 2 ml of distilled water, diluting with 50 ml of glacial acetic acid, and then adding 20 mmol of triphenylphosphine ligand dissolved in 25 ml of glacial acetic acid. Overnight interaction between the green precipitate and the glacial acetic acid solution was observed. After filtering, the catalyst complex was recovered as a dark blue crystal, cleaned with glacial acetic acid, and dried in a desiccator.

Procedure for Synthesis. A three-necked flask (50 ml) containing a magnetic bar was filled with 10 mmol of bis(triphenylphosphine)nickel (II) chloride and 30 mmol of triphenylphosphine. In addition, distilled water (2 ml) and t-butanol (4 ml) were added with a syringe. The mixture was then stirred for 10 minutes at room temperature under an inert nitrogen atmosphere. It was refluxed for two minutes at 80°C. Subsequently, *t*-butanol and H_2O were added in a 2:1 ratio under inert conditions, along with sulfamoyl carboxamides (5a-b) (10 mmol), K_2CO_3 (10 mmol), and a variety of aryl and heteroaryl halides, such as 4-chloroaniline (a), 4-amino-3-chloropyridine (b), and 5-chloro-4,6-diaminopyrimidine (c). It was refluxed for an hour at 100 to 110 degrees

Celsius while being stirred. After cooling it down to room temperature, leucine-based carboxamide derivatives (5c-h) were obtained by recrystallizing it with ethyl acetate and washing it with water. The synthetic route for leucine-based sulfamoyl pentanamide derivatives (**5a-h)** is represented in **scheme 1** while the target compounds were shown in **scheme 2.**

2-{acetyl-[(4-methylphenyl)sulfonyl]amino}-*N*-(4-aminophenyl)-4-methylpentanamide(5c). Yield; 3.19g (95.3%), mp.93-94 ^{o}C, IR (KBr) cm^{-1}: 3373, 3260(N-H), 2800(C-H aliphatic), 1982 (C-H aromatic), 1705, 1690 (C=O), 1684, 1680, 1675 (C=C), 1308,1185(2S=O), 1118(SO_2-NH), 1026(C-N), 723 (Ar-H). ^{1}HNMR (DMSO, 400 MHz) δ: 7.53-7.51 (d, J= 8.0H_2 2H, ArH), 7.46-7.44 (d, J= 8.4H_2, 2H, ArH), 7.18-7.16 (d, J= 8.0 H_2, 2H, ArH), 7.05 -7.03 (d, J= 8.0Hz , 2H, Ar) 3.55 (s,2H, NH_2), 3.54-3.52 (m, IH, NH), 2.48 (s, 3H, CH_3–C=O), 2.16 (s, 3H, CH_3-Ar), 1.44-1.40 (m, 2H, CH), 1.28-1.25 (m, CH, 2CH_3-CH). ^{13}(CNMR (DMSO, 400 MHz) δ: 170. 66, 169.08(C=O), 143.39, 142.93, 139.72, 138.40, 129.72, 129.01, 126.85, 125.84, 123.44, 120.23, 118.54, 116.76 (aromatic carbons), 54.31, 41.17, 39.32, 39.10, 38.84, 38.68, 38.47 (aliphatic carbons). Anal.calcd (%). for $C_{12}H_{27}N_3O_4S$ (417.52): C: 34.49, H: 6.52, N:10.06, S: 7.66. Found: C: 34.50, H: 6.50, N; 10.08, S: 7.68.

2-[Acetyl(phenylsulfonyl)amino*]-N-(*4-aminophenyl)-3-hydroxypropanamide(5d). Yield; 3.20g (95.5%), mp.98-99 ^{o}C,IR (KBr)cm^{-1}: 3369, 3264(2N-H), 2952(C-H aliphatic), 1982(C-H aromatic), 1715, 1679(2C=O), 1323, 1250(2S=O), 1155(SO_2-NH), 940(C=C), 1088 (C-N) 741 (Ar-H). ^{I}HNMR (CD_3CN, 400 MHz), 7.84 (m, 2H, ArH), 7.83 - 7.82 (d, J= 5.2Hz, 2H, ArH), 7.82 -7.81 (d, J= 1.2Hz, 2H, ArH), 7.52 -7.52 (d, J= 1.6Hz, 2H, ArH), 5.97 (s, IH, NH), 4.31 -4.29 (d, J= 5.6H_2, 2H, NH_2) 2.81 (s, IH, CH_3-C=O), 1.69-1.61 (m, IH, CH), 1.48-1.45 (m, 2H, CH_2-CH), 0.88-0.86 (d, J= 6.8Hz, 6H, 2CH_3-CH). ^{13}C-NMR (CD_3CN, 400MHz), 171.45, 170.07, 2 (C=O), 140.41, 132.74, 129.07, 126.91, 117.19, 116.45, 113.64, 112.32, 112.21, 110.54, 109.43, 108.44 (aromatic carbon) 78.27, 77.95, 77.62, 54.21, 41.54, 24.19 (aliphatic carbon). Anal.calcd.(%) for $C_{20}H_{25}N_3O_4S$ (403.50): C: 59.48, H: 6.20, N: 10.41, S: 7.93. Found C: 59.52, H: 6.18, N:10.44, S: 7.89.

2-{acetyl-[(4-methylphenyl)sulfonyl]amino}-*N*-(4-aminopyridin-3-yl)-4-methylpentanamide(5e). Yield; 3.16g (93.7%), mp.91-92 ^{o}C, IR (KBr)cm^{-1}: 3309, 3263(N-H), 2922 (C-H aliphatic) 1982 (C-H aromatic),1711,1660(C=O), 1685(C=N), 1676, 1669(C=C) 1367, 1308(S=O,) 1181, 1118(SO_2N), 1025(C-N), 812(Ar-H). ^{1}HNMR (DMSO, 400 MHz) δ: 6.25-6.25 (d, J= 2.8H_2 2H, ArH), 6.24 (m,2H, ArH), 6.23 (m, IH, ArH), 5.23-5.21 (m, IH, NH), 2.13-4.21 (d, J= 4Hz, 2H, NH_2) , 2.67-2. 64 (m, 3H, CH_3 – C=O), 2.20-2.16 (m, 3H, CH_3-Ar), 1.55 (s, 2H, 2CH), 1.49-1.48 (d, J= 4.8H_2, 6H, 2CH_3). ^{13}C-NMR (DMSO, 400 MHz)δ: 171.61, 170.25(C=O), 156.56(C=N), 129.83, 129.73, 129.73, 129.72, 129.65, 129.62, 129. 46, 127.99, 124.23, 120.57, 117.63 (aromatic carbon) 77.49, 77.17, 76.85, 68.80, 61.86, 33.94, 33.78 (Aliphatic carbons). Anal.calcd (%). for $C_{20}H_{26}N_4O_4S$ (418.51): C, 57.35, H: 6.26, N:13.38, S:7.65. Found: C: 57.37, H: 6.29, N: 13.35, S: 7.67.

2-[Acetyl-[(phenylsulfonyl)amino]-*N*-(4-aminopyridin-3-yl)-4-methylpentanamide(5f). Yield; 3.22g (91.8%), mp.83-84 ^{o}C, IR (KBr)cm^{-1}: 3309, 3231(2N-H), 3063(C-H aliphatic), 1982(C-H aromatic), 1701, 1671(2C=O), 1617 (C=N), 1321-1221(2S=O)1025 (C-N), 935 890 (C=C), 741 (Ar-H). ^{I}HNMR ($CDCl_3$/DMSO, 400 MHz) δ: 7.81(m,2H, ArH), 7.43 (m, 2H, ArH), 7.35(m, IH, ArH), 6.17 (s, IH, NH), 3.61 (s, 2H, NH_2) 2.37 (s, 3H, CH_3-C=O), 0.77 (s, 6H, 2CH_3-CH). ^{1}C-NMR ($CDCl_3$/DMSO, 400 MHz) δ: 172.89, 170.76 (C=O), 157.32 (C=N), 140.10, 132.54, 128.91, 127.09, 128.36, 127.42, 125.85, 123.77, 120.52, 118.96 (aromatic carbon), 77.36, 77.04, 76.73, 55.96, 41.47, 24.26, 22.98, 20.78. Anal.calcd.(%) for $C_{19}H_{24}N_4O_4S$ (404.48): C: 4.70, H: 5.93, N: 13.84, S: 7.91.

Found C: 4.68, H: 5.95, N: 13.80, S: 7.89.

2-{acetyl-[(4-methylphenyl)sulfonyl]amino}-*N*-(4,6-diaminopyrimidin-3-yl)-4-methylpentanamide*e*(5g)

Yield; 3.30 g (95.5%), mp.107-108 ^{o}C, IR (KBr)cm^{-1}: 3328, 3130(2N-H), 3003(C-H aliphatic) 1986 (C-H aromatic), 1713, 1660(C=O), 1680, 1678(C=N), 1367, 1278 (S=O), 1118 (SO_2N), 1088(C-N), 970 (C=C), 890 (Ar-H). ^{1}HNMR (DMSO 400 MHz) δ: 7.81 (d, J = 7.3Hz, 2H, ArH), 7.59-7.53 (m, 2H, ArH), 7.36 (d, J=7.0Hz, IH, ArH) 2.48 (S,IH, NH), 2.36 (s, 2H , NH_2), 2.27 (s,3H, CH_3-C=O), 2.23 (s, 3H, CH_3-Ar), 1.34-1.32 (m, 2H, 2CH), 1.30 (s, 6H, $2CH_3$). ^{13}CNMR (DMSO, 400 MHz) δ: 170.96, 169.35 (C=O), 156.88, 158.79, 142.19, 139.75, 138.15, 130.64, 129.43, 129.37, 128.78, 126.95 (aromatic carbons), 78.92, 78.59, 78.27, 54.21, 41.49, 40.05, 39.84 (aliphatic carbons). Anal.calcd (%). for $C_{19}H_{26}N_6O_4S$ (434.51): C, 52.47, H, 6.03, N, 19.33, S, 7.36. Found: C: 52.49, H: 6.05, N:19.30, S:7.35.

2[Acetyl(phenylsulfonyl)amino*]-N-(*4,6-diaminopyrimidin-5-yl)-4-methylpentanamide(5h).

Yield; 3.18 g (94.6%), mp.100-101 ^{o}C, IR (KBr) cm^{-1}: 3341 (N-H), 3063 (C-H aliphatic), 1982 (C-H aromatic), 1711, 1679(2C=O), 1630, 1580 (C=N), 1580 (N-H), 1308, 1155 (2S=O), 1088 (SO_2-NH), 1025 (C-N), 995 (C=C) 894 (Ar-H). ^{I}HNMR(DMSO, 400 MHz)δ: 8.51-8.49(m, 2H, ArH), 7.16-7. 15 (m, 2H, ArH), 7.15-7.14(m, IH, ArH), 4.35 (s, IH, NH), 3.34 (s, 4H, $2NH_2$), 2.48 (s, IH, CH), 2.47-2.47 (m, 2H, CH_2-CH), 2.18 (m, IH, CH), 1.79 (s, 6H, $2CH_3$CH). ^{13}CNMR (DMSO, 400 MHz)δ: 170.33, 170.09, 2(C=O), 166.77, 154.89 (C=N), 146.15, 130.64, 129.43, 129.37, 128.78, 126.95, 125,79, 124.77, 121.99, 119.43 (aromatic carbons), 48.76, 40.55, 40.34, 40.13, 39.93, 39.50 (aliphatic carbon). Anal.calcd.(%) for $C_{18}H_{24}N_6O_4S$ (420.49): C: 51.37, H;5.71, N: 19.98, S: 7.61. Found C: 51.41, H: 5.68, N: 19. 95, S: 7.58

Biological Evaluations

Antimicrobials studies. The antibacterial screening of each compound was conducted using the Agar dilution technique.[22] Clinical isolates from the pharmaceutical microbiology and biotechnology labs at the University of Nigeria, Nsukka, Nigeria, were used to screen them for in vitro antimicrobial activity against microbes, including Salmonella typhi, Aspergillus niger, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, and Escherichia coli. Using 0.5 McFarland turbid equivalents, the organisms were standardized. For the antibacterial and antifungal analyses, ofloxacin was the standard, and fluconazole was utilized. **Table 1** lists the different minimum inhibitory concentrations for the standards and tested compounds.

Antioxidant Studies

Antioxidant Activity: The antioxidant assessment was performed using the Blois technique.[23] 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical inhibition was used to measure the antioxidant potential of the compounds in vitro.

Physicochemical studies

The compounds' physicochemical characteristics were determined in silico. Topological surface area (TPSA), molecular weight (MW), octanol/water partition coefficient logP(o/w), aqueous solubility (SlogP), number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), number of rotatable bonds (NRB), and other physicochemical data were obtained. We computed these physicochemical characteristics using the descriptors calculator found at Swiss Dock internet servers. The compounds' potential for use as drugs was evaluated using Lipinski's rule of five.

Molecular Docking Protocol

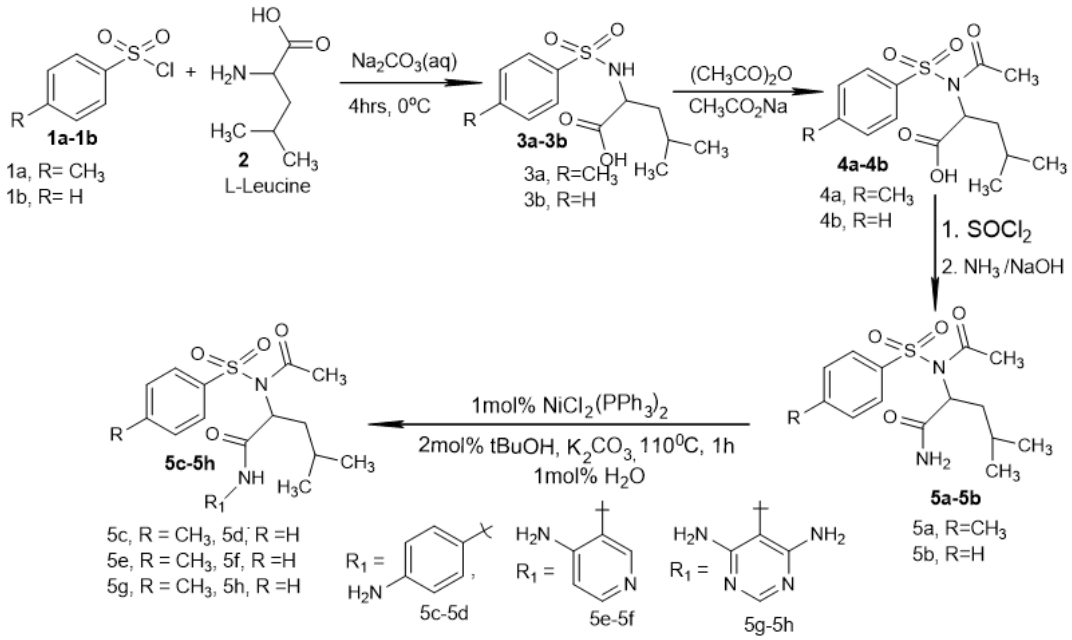
The molecular docking scores of the eight compounds that interact with the target receptors for antifungal (PDB code: 1WS3), antioxidant (PDB code: 1HD2), and antibacterial (PDB code: 5MMN) were investigated.[24,25] To realize this idea, protein preparation was done using Biovia Discovery Studio. This was achieved by extracting the water molecules from the proteins and then identifying

and modifying the binding sites to incorporate a large fraction of the active sites found in the proteins. Moreover, the generated proteins were supplemented with polar hydrogens and saved in PDB format. Additionally, the Autodock Vina program[26,27] was used to carry out the proper molecular docking analysis between the target receptor and the synthesized compounds (5a, 5b, 5c, 5d, 5e, 5f, 5g, and 5h). The PDBQT formatted proteins and ligands, along with the Vina, licensing, and split files, were meticulously transferred into their respective working directories, along with the conf.txt file. In addition, the configuration files were opened, and for every working folder, the receptor, ligand, coordinates, radius, and exhaustiveness were set. As a summary, the vina.exe application was used, the working directories holding the files were copied into the command prompt accordingly, the docking process was completed, and binding affinities were created. To find the ideal position of the ligand-protein interactions, the output files were then divided into sets of exhaustiveness. The Biovia discovery studio was utilized to do further 2D and 3D visualization of the docking score.

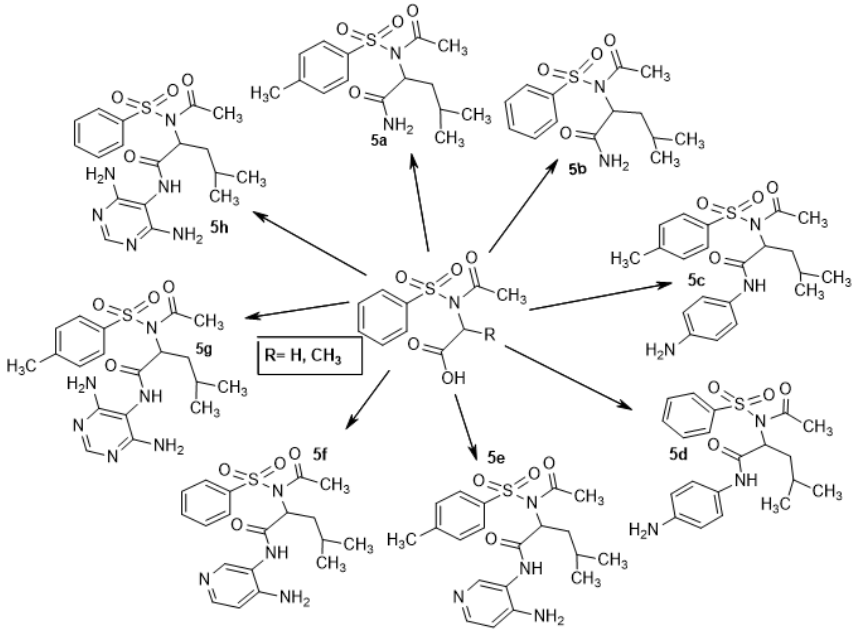
RESULTS AND DISCUSSION

Spectra

The sulfamoyl carboxamides displayed their N-H and S=O bands in the 1367–1151 cm–1 and 3373–3257 cm–1 frequency range, respectively, in their FTIR spectra. Carboxamide bands with a C = O were seen between 1690 and 1660 cm-1; these bands showed that pentanamides and sulfonamides had successfully coupled. The successful synthesis of the target compounds was supported by the 1H-NMR peaks between 6.174 and 2.125 ppm. The synthesis of sulfamoyl pentanamides was indicated by the C=O peaks in the 13C-NMR, which were located between 172.898 and 169.0 ppm. The carbon-13 NMR analysis revealed the presence of all the aromatic and aliphatic carbon peaks. The elemental analysis took the compounds' elemental compositions into consideration.



Scheme 1: Synthetic route for leucine-based sulfamoyl pentanamide derivatives



Scheme 2: Lucine-based Sulfamoyl pentanamide derivatives

Biological Studies

Antimicrobial Activities.

The results in table **1** show that all the target compounds exhibited significant antimicrobial activities. Generally, compounds **5g** exhibited the best antibacterial activities being the only compound that inhibited the growth of all the test bacteria, namely *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Salmonella typhi* while compounds **5c** and **5e** were the best antifungal agents having inhibited the growth of all the test fungi namely *Candida albicans and Aspergillus niger*. Specifically, compounds **5f** (MIC 0.40mg/ml) and **5h** (MIC 0.40gm/ml) were the most potent against *E.coli* while compound **5f**(MIC 0.30mg/ml and 0.40gm/ml) was found to be the most active antibacterial agent against *S.typhi, S.aureus and B.sub* respectively. Novelty was recorded in the antimicrobial studies, it was previously reported that sulfonamides do not inhibit the growth of *Pseudomonas aeruginosa* a recalcitrant bacterium[28-31], however, compounds **5a** (MIC 0.60mg/ml) and **5g**(MIC 0.70mg/ml) exhibited a significant inhibitory activities against *Pseudomonas aeruginosa* and this could be attributed to synergism in microbial antagonism arising from the combination of sulfonamide, carboxamide and leucine moieties[32] in a single drug compound. The antifungal studies revealed that *Aspergillus niger* resisted many of the target compounds because sulfonamides scarcely inhibit the growth of *Aspergillus niger* [33,34], nevertheless, compounds **5c** (MIC 0.70mg/ml) and **5e** (MIC 0.50mg/ml) displayed considerable antifungal activities against the recalcitrant fungus. Similarly, compound **5a**(MIC 0.70mg/ml) was found to be the most potent antifungal agent against *Candida albican.*

The antimicrobial mechanism of sulfonamides has shown that they imitate the substrate para-aminobenzoic acid (PABA), which bacteria utilize to synthesis folic acid, hence engaging in competitive inhibition. Bacteria cannot properly synthesis folic acid when they absorb

sulfonamides in place of PABA, which interferes with vital metabolic processes. Certain amino acids and nucleic acids (DNA and RNA) cannot be produced without folic acid. Consequently, sulfonamides stop bacteria from growing and eventually kill them by blocking the production of folic acid. Interestingly, sulfonamides are known to have selective toxicity, which means that while they primarily affect bacterial cells, they seldom affect human cells at all. This is due to the fact that folic acid is derived from food rather than being synthesized by human body. Thus, sulfonamides have no effect on the synthesis of folic acid in human cells. Furthermore, owing to their broad-spectrum activity, sulfonamides have the ability to effectively combat a broad spectrum of bacteria, which makes them useful in the treatment of a number of diseases. However, the specific type of bacteria and their sensitivity to sulfonamide drugs determine how successful the treatments are.[28,35]

The structure activity relationship (SAR) study of the compounds for antimicrobial activity showed that both *p*-toluenesulfonamide and benzenesulfonamide derivatives possess significant antimicrobial activity. This underscores the potency of sulfonamide moiety as an antimicrobial agent. However, compounds bearing *p*-toluenesulfonamide moiety (**5a**, **5c, 5e** and **5g**) displayed broader spectrum of antimicrobial activity. This may be attributed to the presence of an electron-donating methyl group at position 4 of the phenyl ring of *p*-toluenesulfonamide moiety which gives it a closer structural resemblance to *p*-aminobenzoic acid (PABA), a compound required by bacteria for the synthesis of folic acid, and enables it to mimick PABA better than benzenesulfonamide analogues. Furthermore, the presence of *p*-toluenesulfonamide moiety improved antimicrobial potency of the compounds containing it as this moiety was found to be the only moiety common to the compounds (**5a** and **5g**) that inhibited the recalcitrant bacterium *Pseudomonas aeruginosa* known for its resistance to sulfonamide[28]. Amongst these compounds containing *p*-toluenesulfonamide moiety, the introduction of diaminopyrimidine ring (**5g**) further broadened the antibacterial activity as compound **5g** was the only compound that inhibited the growth of all the bacteria used in the antibacterial evaluation. Amongst the benzenesulfonamide analogues (**5b**, **5d**, **5f** and **5h**), the presence of aminopyridine ring (**5f**) improved the antimicrobial potency as **5f** exhibited the best MIC (≤0.40 mg/ml), almost comparable to ofloxacin.

Table 1: Minimum inhibitory concentration (mg/ml)

Sample no	*E.coli*	*S.typhi*	*S.aureus*	*B. sub*	*Ps.aerug*	*C.albicans*	*A. niger*
5a	0.90	0.80	-	0.80	0.60	0.70	-
5b	0.70	0.70	0.90	0.60	-	-	-
5c	0.70	1.00	0.90	0.60	-	0.90	0.70
5d	0.70	0.60	0.60	0.60	-	-	-
5e	0.80	1.00	0.90	0.60	-	0.90	0.50
5f	0.40	0.30	0.30	0.40	-	-	-
5g	0.80	0.90	0.50	0.50	0.70	0.80	-
5h	0.40	0.50	0.50	0.50	-	-	-
Ofloxacin	0.05	0.05	0.01	0.02	0.03	-	-
Fluconazole	-	-	-	-	-	0.02	0.05

Key: - implies no activity. Ofloxacin was the antibacterial reference drug and Fluconazole was the antifungal standard drug used.

Antioxidant Activities

The antioxidant activity of substances can be classified as weak (IC50 = 250–500 µg/ml), moderate (IC50 = 101–150 µg/ml), extremely strong (IC50 <50 µg/ml), and strong (IC50 = 50–100 µg/ml), according to Setha et al.[36] Table 2 illustrates this concept, demonstrating that every molecule had potent antioxidant properties that allowed it to either stop or slow the development of oxidative stress and the illnesses it causes. The compounds 5g (IC50 1.174µg/ml) and 5h (IC50 1.172µg/ml) demonstrated antioxidant properties at a concentration of 200µg/ml, which was equivalent to ascorbic acid (IC50 1.000µg/ml). It is worthy to note that lower IC_{50} value indicates better antioxidant potential and based on that principle, compound **5h** having the lowest IC_{50} value of 1.172µg/ml was found to be the best antioxidant agent synthesized. Similar compounds exhibited considerable antioxidant activities.[37] The implication is that compound **5h** can serve as an antioxidant agent comparable with ascorbic acid. Stable DPPH free radicals have the ability to react with compounds that donate hydrogen atoms and therefore this assay is always employed in the measurement of the reducing ability of antioxidants towards stable DPPH free radicals. Beyond scavenging of free radicals, sulfonamides have been found to activate NRF2 an endogenous antioxidant[18] and the use of amino acids as precursors are likely to enhance their antioxidant activities.[38]

The SAR study of the compounds (**5a-5h**) as antioxidant agents, revealed that compounds containing benzenesulfonamide moiety (**5b**, **5d, 5f** and **5h**) possess better antioxidant properties that *p*-toluenesulfonamide derivatives based on the IC_{50} values. This may be attributed to the observation that the addition of electron donating group ($-CH_3$) to the benzene ring decreased the antioxidant activities of the compounds. The introduction of aminopyrimidine ring to both *p*-toluenesulfonamide and benzenesulfonamide derivatives resulted in improved antioxidant activity as the analogues bearing aminopyrimidine ring (**5g** and **5h**) exhibited the best antioxidant activities comparable to ascorbic acid.

Table 2: *In vitro* antioxidant (% scavenging activity)

Sample no	% inhibition at 200 µg/ml	% inhibition at 100 µg/ml	% inhibition at 50 µg/ml	IC_{50} (µg/ml)
5a	74.79	72.22	73.44	1.352
5b	52.38	50.12	55.49	1.928
5c	29.67	45.85	15.57	3.939
5d	88.10	40.66	39.68	1.754
5e	56.72	38.83	26.80	2.133
5f	24.91	63.98	83.15	**1.825**
5g	85.60	87.06	80.04	1.174
5h	85.58	87.08	80.06	1.172
Ascorbic acid	96.83	97.68	97.31	1.000

Key: The standard antioxidant drug = ascorbic acid.

Prediction of Drug-likeness and Oral Bioavailability

Table 3 shows the target compounds' physicochemical characteristics. Lipinski's rule of five[39] stipulates that a drug molecule is considered drug-like if it meets specific requirements, including lipophilicity (logP) < 5, molecular weight (MW) ≤ 500, number of hydrogen bond donor (HBD) ≤ 5, and number of hydrogen bond acceptor (HBA)

≤ 10. Furthermore, in accordance with Verber's principle, drug molecules with a topological polar surface area (TPSA) of ≤140 Å2 can enter mammalian cells and demonstrate good oral bioavailability, while TPSA ≤ 90 Å2 indicates that the drug molecule can also enter the blood-brain barrier (BBB) and the central nervous system (CNS). This constitutes a surrogate property for cell permeability. Moreover, it was shown that NRB ≤10 is necessary for adequate oral bioavailability.[40,41] All the compounds demonstrated strong oral bioavailability and outstanding drug-likeness, in accordance with the aforementioned guiding criteria. While compounds 5a–h have high oral bioavailability and can permeate cells, none of the compounds can cross the blood-brain barriers or the central nervous system.

Table 3: Physicochemical properties of compounds

Sample no	HBA	HBD	NRB	logP(o/w)	SlogP	TPSA	MW	Lip violation
5a	4	1	7	1.90	1.43	97.54	326.41	0
5b	4	1	7	1.60	1.12	97.54	312.39	0
5c	4	2	8	3.23	3.17	109.57	417.52	0
5d	4	2	8	2.94	2.86	109.57	403.50	0
5e	5	2	8	2.00	2.56	122.46	418.51	0
5f	5	2	8	1.70	2.25	122.46	404.49	0
5g	6	3	9	0.99	1.54	161.37	434.51	0
5h	6	3	9	0.69	1.23	161.37	420.49	0

Molecular Docking

In recent years, molecular docking has been widely used to investigate the drug's ability of molecules on a global basis, as it seeks to explain several modules underlying Protein-ligand interaction, which corroborate the process of proteins interacting with ligands to form stable complexes with biologically significant functions.[41] Protein-ligand complexes, on the other hand, play an important role in a wide range of biological activities. Ligands are bound to proteins by intermolecular interactions such as ionic bonds, hydrogen bonds, and van der Waals forces. As a result, particular protein-ligand interaction types are essential to understand protein function. As a result, eight synthesized compounds were investigated and compared by docking with three disease conditions namely; bacterial infections (antibacterial activity), fungal infections (antifungal activity) and oxidative stress (antioxidant activity) are to be studied and the following drug targets were selected for molecular docking studies. The target receptor for antibacterial is *Escherichia coli* DNA gyrase in complex with 1-ethyl-3-[8-methyl-5-(2-methyl-pyridin-4-yl)-isoquinolin-3yl]urea (PDB code: **5MMN**); antifungal is urate oxidase from *Aspergillus flavus* complexed with uracil (PDB code: **1WS3**), and for antioxidant is human peroxiredoxin 5 (PDB code: **1HD2).** These target receptors were chosen based on the protein data's predictability in light of being possibly effective for binding to the suggested treatment, as each of the proteins has several active sites. Additionally, examinations between the ligands and proteins were examined in order to suggest or postulate which matches better for compatibility in terms of combating these disease conditions.

Substantially, the antifungal target receptors revealed very important biological properties upon interacting with the eight synthesized compounds, thus implying promising compatibilities. As presented in **table 4** and **figure S1**, the significant binding affinities ranged from -6.9kcal/mol to -

8.0 kcal/mol. As a result, **5g**@1WS3 calculated the best binding affinity of -8.0 kcal/mol, as further validated by the conventional hydrogen bonds incorporated as amino acid residues (ARG C: 108, THR C: 107, CYS C: 103). This was followed by **5f**@1WS3 and **5c**@1WS3, which had a comparable binding affinity of -7.8 kcal/mol, as well as **5e**@1WS3 and **5h**@1WS3, which had a binding affinity of -7.7 kcal/mol. Very interesting information noticed in the said interactions, is the fact that all interactions displayed similar conventional hydrogen bonds embedded as arginine, tryptophan, and threonine. Fascinatingly, the superiority of the investigated interactions can be derived in the following order: **5g**@1WS3 > **5c**@1WS3 > **5f**@1WS3 > **5e**@1WS3 > **5h**@1WS3 > **5d**@1WS3 > **5a**@1WS3 > **5b**@1WS3. On the other hand, the antibacterial potentials of the synthesized compounds were examined to suggest very minimal efficiency upon interaction with the antibacterial target receptors. As evident from **table 4** and **figure S2**, it can be observed that **5h**@5MMN calculated the most significant binding affinity of -6.9 kcal/mol, thus elucidating the conventional hydrogen bonds to be embedded as glutamine and arginine. Additionally, **5g**@5MMN further demonstrated GLY A:77, GLU A:50 and ASN A:46 to be its key pockets for the antibacterial studies, as it also calculated -6.6 kcal/mol as its binding affinity. Interestingly, **5f**@5MMN and **5a**@5MMN revealed similar binding affinity of -6.3 kcal/mol. Overall analysis of the antifungal investigation, shows that the binding affinity ranged from -5.6 kcal/mol to -6.9 kcal/mol. Contrary to the aforementioned disease conditions (fungal and bacterial infections) discussed, the antioxidant potentials of the synthesized molecules as evident in **table 4** and **figure S3**, suggest that only **5a**@1HD2, **5c**@1HD2, **5d**@1HD2, and **5h**@1HD2 revealed favourable conventional hydrogen bonds hence calculating relatively weak binding affinities of -3.9 kcal/mol, -4.2 kcal/mol, -4.0 kcal/mol, and -4.2 kcal/mol.

Summarily, from this investigation, it can be postulated that the eight synthesized compounds were found to elucidate efficient antifungal and antibacterial potentials, as recorded based on their electrostatic forces, electrodynamic forces, and binding affinities, which establishes the interactions formed when atoms of various particles approach into close proximity to one another and alter one another's reactivity. However, most of the compounds were found to possess comparatively significant antibacterial, antifungal and antioxidant potentials. Thus, implying them to be potential compounds for the treatment of both bacterial and fungal infections and less in considering antioxidant potentials. Generally, compounds bearing sulfonamide and carboxamide functionalities have been found to exhibit broad spectrum of biological activities[43,44], and molecular docking has been very useful in ascertaining their suitability as drug candidates [45-49].

CONCLUSION

In this paper, we have reported a facile and efficient approach to the synthesis of leucine-based sulfamoyl pentanamide derivatives. Generally speaking, the *in vitro* biological studies revealed that compounds **5a, 5f, 5g and 5h** were the best antibacterial agents according to their minimum inhibitory concentrations, compounds **5a, 5c, 5e** and **5g** exhibited the best antifungal activities while compounds **5g** and **5h** were found to be the best antioxidants. Furthermore, the molecular docking studies showed the binding affinity of the compounds mentioned above in the order **5h** > 5g > **5f** > 5a for antibacterial potential, 5g > 5c > 5e > 5a for antifungal potential and 5g > 5g for antioxidant potentials. In the next phase of this research, in vitro enzyme assay, in vivo analysis and other relevant assessments would be included for more comprehensive understanding of the biological activities of target compounds. The physicochemical parameters evaluations confirmed all the compounds to be likely drugs that would not pose oral bioavailability problems having satisfied Lipinski's rule of five. All the target compounds are potential antimicrobial and antioxidant agents.

Table 4: Antifungal, antibacterial and antioxidant molecular docking analysis

Compounds	Best pose (Binding affinity (Kcal/mol)			Nature of interactions		
	Antifungal	antibacterial	antioxidant	antifungal	antibacterial	antioxidant
5a	-7.2	-6.3	-3.9	THR C:107, TRP C:106	A:ARG:76, A:GLY770	A:BEZ:201, A:ILE:119
5b	-6.9	-5.6	-0.0	TRP C:106, THR C:107	A:ASN:46, A:ARG:76	Unfavourable bonds
5c	-7.8	-6.0	-4.2	TRP C:106, THR C:107, ARG C:108	A:THR:46	BEZ A:201, PRO A:45, LEU A:116
5d	-7.4	-6.1	-4.0	VAL C:73, MET C:32	A:ARG:76, A:GLU:50, A:ASP:73	A:ILE:119, A:BEZ:201
5e	-7.7	-6.1	-3.8	ARG C:128, GLU C:31, THR C:74	A:GLY:77, A:GLU:50, A:ASN:46	Unfavourable bonds
5f	-7.8	-6.3	-4.2	TRP C:106, VAL C:29, ARG C:108	A:GLY:77, A:GLU:50, A:ASN:46	Unfavourable bonds
5g	-8.0	-6.6	-4.4	ARG C: 108, THR C: 107, CYS C: 103	A:GLU:50, A:ASN:46	Unfavourable bonds
5h	-7.7	-6.9	-4.2	THR A: 143, ARG C:74	A:GLU:50, A:ARG:136,A:ARG:76	A:PHE:43, A:ILE:119

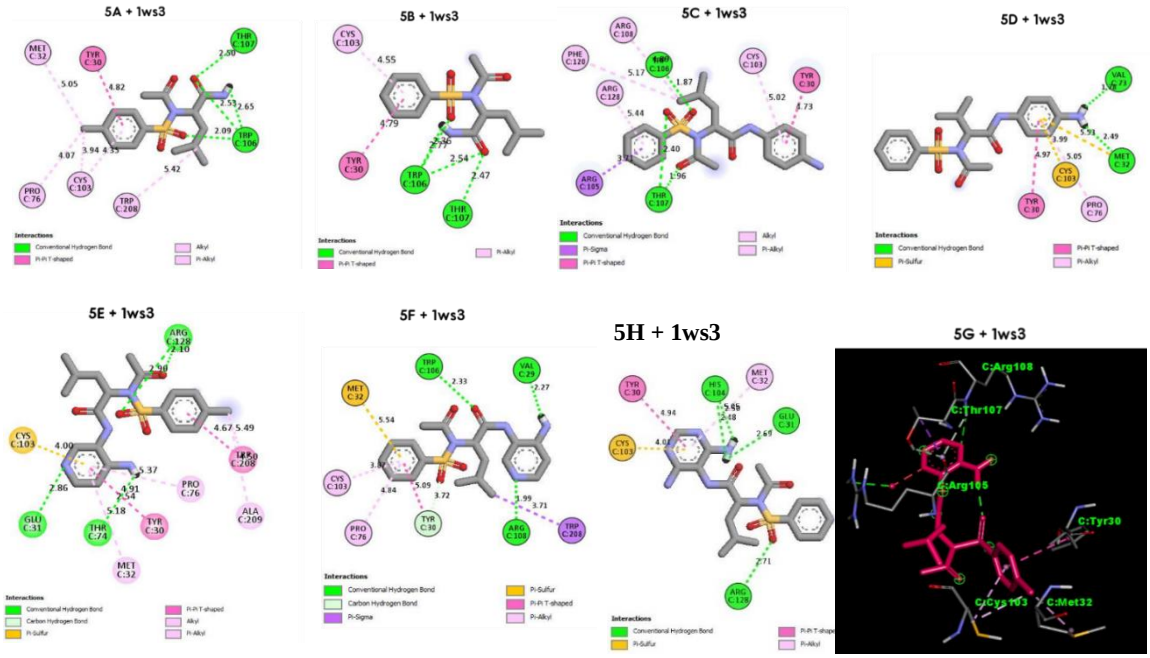


Figure S1: 3D visualization of the antifungal interaction using 1WS3

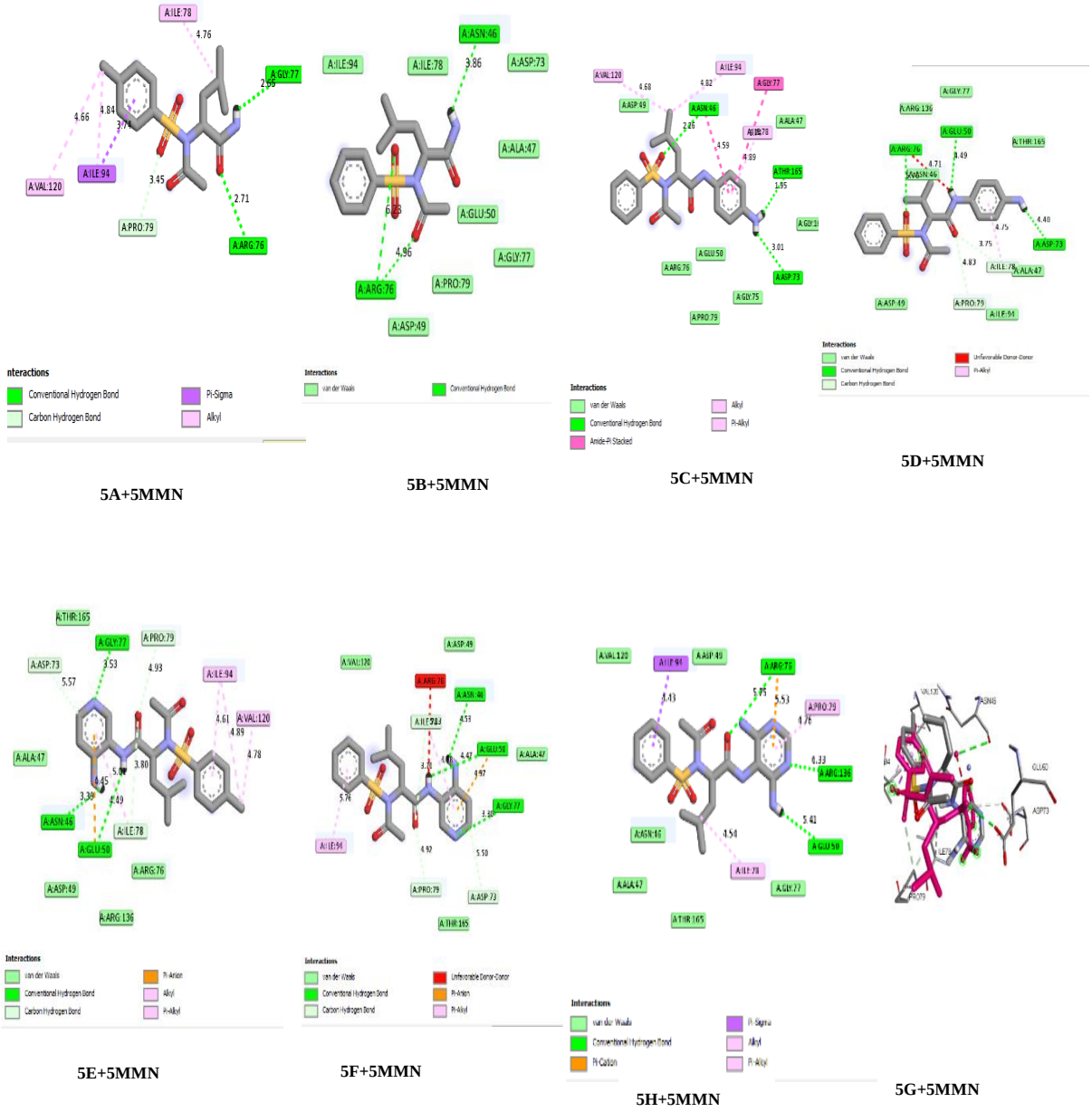


Figure S2: 3D visualization of the antibacterial interactions using 5MMN

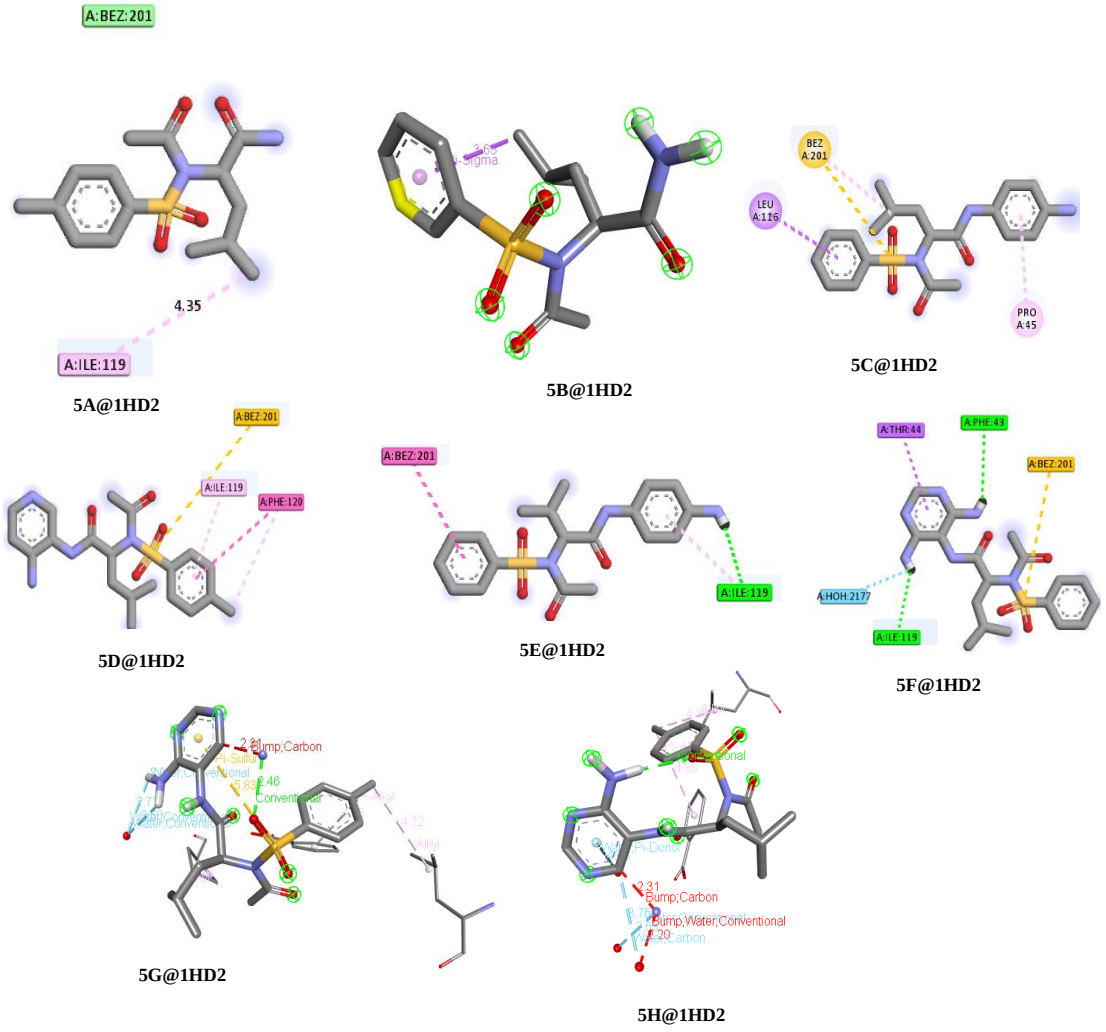


Figure S3: 3D visualization of the antioxidant interactions using 1WS3

ACKNOWLEDGEMENT

Mrs. Ekianinima Okonny-Egbujor is acknowledged for her support during this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FINANCIAL DISCLOSURE

The authors declare that no financial aid was received from any external body.

REFERENCES

1. Abu Taha A. Spectrum and Antibiotic Resistance in the Community and Hospital-Acquired Urinary Tract Infected Adults. *Jordan J Pharm Sci*. 2023; *16*(2):455. https://doi.org/10.35516/jjps.v16i2.1494
2. Tai J., Shin J-M., Park J., Han M. and Kim T.H. Oxidative stress and antioxidants in chronic rhinosinusitis with nasal polyps. *Antioxidants*. 2023; 12:195. DOI: https://doi.org/10.3390/antiox12010195
3. Egbujor M.C., Okoro U.C., Egu A.S., Okonkwo V.I., Okafor S.N., Emeruwa C.N., et al. Synthesis and biological evaluation of sulfamoyl carboxamide derivatives from sulfur-containing α-amino acids. *Chiang Mai J. Sci.* 2022; 49: 1100–1115 DOI: https://doi.org/10.12982/CMJS.2022.070
4. Meunier B. Hybrid Molecules with a Dual Mode of Action: Dream or Reality. *Acc Chem Res*. 2009; 41:69-77. DOI: https://doi.org/10.1021/ar7000843
5. Chugunova E.A. and Burilov A.R. Novel Structural Hybrids on the Base of Benzofuroxans and Furoxans. Mini-Review. *Curr Top Med Chem*. 2017; 17: 986–1005. DOI: https://doi.org/10.2174/1568026616666160927145822.
6. Egbujor M.C. and Okoro U.C. New methionine-based p-toluenesulphonamoyl carboxamide derivatives as antimicrobial and antioxidant agents: design, synthesis and molecular docking. *J. Pharm. Res. Int*. 2019; 28:1-12. DOI: https://doi.org/10.9734/jpri/2019/v28i130192
7. Egbujor M.C., Okoro U.C., Okafor S. and Nwankwo N.E. Design, synthesis and molecular docking of novel serine-based sulphonamide bioactive compounds as potential antioxidant and antimicrobial agents. *Indo Am J Pharm Sci*. 2019; 06:12232–12240. DOI: https://doi.org/10.5281/zenodo.3250306
8. Egbujor M.C., Okoro U.C., Emeruwa C.N., Umeh O.R., Eziafakaego M.I., Egwuatu P.I., et al. Synthesis of Sulphonamides Using Threonine, and Evaluation of Their Biological Activities; in Rizvi S.A.A., ed., *Challenges and Advances in Pharmaceutical Research*, Vol. 2. P.B. International, India, 2022; 95–107. https://doi.org/10.9734/bpi/capr/v2/2274E.
9. Fox S.W., Fling M. and Bollenback G.N. Inhibition of bacterial growth by d-leucine. *J. Biol. Chem*. 1944; 155:465-468. DOI: https://doi.org/10.1016/S0021-9258(18)51176-5.
10. Brunati M., Bava A. and Lancini G. Influence of leucine and valine on ramoplanin production by Actinoplanes sp. ATCC 33076. *J Antibiot*. 2005; 58:473-478. DOI: https://doi.org/10.1038/ja.2005.63.
11. Jin H.J., Lee J.H., Kim H.H., Kim K-T., Lee G.W., Choi S.J., Chang, P-S. and Park H-D. Antioxidative and nitric oxide scavenging activity of branched-chain amino acids. *Food Sci Biotechnol*. 2015; 24:1555-1558. DOI: https://doi.org/10.1007/s10068-015-0200-2.
12. Egbujor M.C., Okoro U.C., Okafor S.N., Amasiatu I.S., Amadi U.B. and Egwuatu P.I. Synthesis, molecular docking and pharmacological investigation of some 4-methylphenylsulphamoyl carboxylic acid analogs. *Int. J. Res. Pharm. Sci*. 2020;11:5357-5366. DOI: https://doi.org/10.26452/ijrps.v11i4.3157.
13. Egbujor M.C., Okoro U.C. and Okafor S. Design, synthesis, molecular docking, antimicrobial, and antioxidant activities of new phenylsulfamoyl carboxylic acids of pharmacological interest. *Med. Chem. Res*. 2019; 28:2118-2127. DOI: https://doi.org/10.1007/s00044-019-02440-3.
14. Egbujor M.C., Okoro U.C., Okafor S. and Nwankwo N.E. Synthesis, characterization, and in silico studies of novel alkanoylated 4-methylphenyl sulphonamoyl carboxylic acids as potential antimicrobial and antioxidant agents. *Int. J. Pharm. Phytopharm. Res*. 2019; 9:89-97.

15. Raul S.J., More A.H. And Mahajan S.S. *Intl. J. of Res Pharm Chem.* 2011; 1(4): 991.
16. Egbujor M.C., Egu S.A., Okonkwo V.I., Jacob A.D., Egwuatu P.I. and Amasiatu I.S. Antioxidant drug design: historical and recent developmentsj. *Pharm. Res. Int*. 2021; 32:36-56. DOI: https://doi.org/10.9734/jpri/2020/v32i4131042.
17. Badgujar J.R., More D.H. and Meshram J.S. Synthesis, antimicrobial and antioxidant activity of pyrazole-based sulfonamide derivatives. *Indian J Microbiol*. 2018; 58:93-99. DOI: https://doi.org/10.1007/s12088-017-0689-6.
18. Egbujor M.C., Garrido J., Borges F. and Saso L. Sulfonamide a valid scaffold for antioxidant drug development. *Mini Rev Org Chem.* 2023; 20:190-209. DOI: https://doi.org/10.2174/1570193X19666220411134006.
19. Egbujor M.C., Petrosino M., Zuhra K. and Saso L. The role of organosulfur compounds as nrf2 activators and their antioxidant effects. *Antioxidants*. 2022; 11:1255. DOI: https://doi.org/10.3390/antiox11071255.
20. Bhole R., Zambare Y. and Bonde C. Synthesis and evaluation of novel n-cycloheptyl-substituted -2,3-dihydro-1,3- benzothiazole-2-carboxamide targeting the estrogen binding receptor, *Jordan J Pharm Sci*. 2019; 12(3).
21. Venanzi L.M. Tetrahedral complexes of nickel (II) and the factors determining their formation. *J. Chem. Soc.* 1958; 8:137-142. DOI: https://doi.org/10.1016/0022-1902(58)80175-x.
22. Wiegand I., Hilpert K. and Hancock R.E.W. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Protoc*. 2008; 3:163-175. DOI: https://doi.org/10.1038/ nprot.2007.521.
23. Blois M.S. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*. 1958; 181:1199–1200. DOI; https://doi.org/10.1038/1811199a0.
24. Panchaud P., Bruyere T., Blumstein A. C., Bur D., Chambovey A., Ertel E. A. and Rueedi G. Discovery and Optimization of Isoquinoline Ethyl Ureas as Antibacterial Agents. *J Med Chem*. 2017; 60:3755-3775. DOI: https://doi.org/10.1021/acs.jmedchem.6b01834.
25. Retailleau P., Colloc'h N., Vivares D., Bonneté F., Castro B., El Hajji M. and Prangé T. Urate oxidase from Aspergillus flavus: new crystal-packing contacts in relation to the content of the active site. *Acta Crystallogr D Biol Crystallogr*. 2005; 61:218-29. DOI; https://doi.org/10.1107/S0907444904031531.
26. Evrard C., SA-Namur B. V., Clippe A., Bernard A. and Knoops B. Crystal structure of human peroxiredoxin 5, a novel type of mammalian peroxiredoxin at 1.5 A resolution. *J Mol Biol. 2001*; 311:751-9. DOI: https://doi.org/10.1006/jmbi.2001.4853.
27. Trott O. and Olson A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010; 31(2):455–461. DOI: https://doi.org/10.1002/jcc.21334.
28. Egbujor M.C., Nwobodo D.C., Egwuatu P.I., Abu I.P. and Ezeagu C.U. Sulphonamide drugs and pseudomonas aeruginosa resistance: a review. *Int. J. Modern Pharm. Res*. 2020; 4:78-83.
29. Egbujor M.C., Okoro U.C., Egu S.A., Egwuatu P.I., Eze F.U. and Amasiatu I.S. Synthesis and biological evaluation of alanine derived bioactive p-toluenesulphonamide analogs. *Int. J. Res. Pharm. Sci*. 2020; 11:6449-6458. DOI: https://doi.org/10.26452/ijrps.v11i4.3440.
30. Miller-Hjelle M.A., Somaraju V. and Hjelle J.T. Modern Pharmacology with Clinical Applications/edited by C. R. Craig, R.E. Stitzel, (6th ed). *Philadelphia: Lippincott Williams & Wilkins*. 2004. DOI: https://trove.nla.gov.au/version/46530718.

31. Zessel K., Mohring S., Hamscher G., Kietzmann M. and Stahl J. Biocompatibility and antibacterial activity of photolytic products of sulfonamides. *Chemosphere.* 2014; 100:167-174.
DOI: https://doi.org/10.1016/j.chemosphere.2013.11.038.
32. Egbujor M.C., Okoro U.C. and Okafor S. Novel alanine-based antimicrobial and antioxidant agents: synthesis and molecular docking. *Indian J. Sci. Technol.* 2020; 13:1003-1014.
DOI: https://doi.org/10.17485/ijst/2020/v013i09/146687.
33. Mahmoud I., Altaif K., Sini M.A., Daoud S. and Aqel N. Determination of antimicrobial drug resistance among bacterial isolates in two hospitals of Baghdad. *Jordan J Pharm Sci. 2020; 13(1).*
34. Egbujor M.C., Okoro U.C., Okafor S.N., Egu S.A., Amasiatu I.S., Egwuatu P.I., et al. Design, synthesis, and molecular docking of cysteine-based sulphonamide derivatives as antimicrobial agents. *Res Pharm Sci*. 2021; 17:99-110.
DOI: https://doi.org/10.4103/1735-5362.329930.
35. Ovung A. and Bhattacharyya J. Sulfonamide drugs: structure, antibacterial property, toxicity, and biophysical interactions. *Biophys Rev.* 2021; 13(2):259-272.
doi: https://doi.org/10.1007/s12551-021-00795-9
36. Setha B., Gaspersz F., Febe F., Idris A.P.S., Rahman S. and Mailoa M.N. Potential of seaweed padina sp. as a source of antioxidant. *Int. J. Sci. Tech. Res*. 2013, 26:221.
37. Egbujor M.C., Okoro U.C., Nwobodo D.C., Ezeagu C.U., Amadi U.B., Okenwa-Ani C.G., et al. Design, Synthesis, Antimicrobial and Antioxidant Activities of Novel Threonine-based Sulfonamide Derivatives. *J. Pharm. Res. Int.* 2020; 32:51-61.
DOI: https://doi.org/10.9734/jpri/2020/v32i830470.
38. Egbujor M.C., Olaniyan O.T., Emeruwa C.N., Saha S., Saso L. and Tucci P. An insight into role of amino acids as antioxidants via NRF2 activation. *Amino Acid.* 2024; 56:23.
Doi: https://doi.org/10.1007/s00726-024-03384-8
39. Lipinski C.A., Lombardo F., Dominy B.W. and Feeney P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2001; 46:3-26.
DOI: https://doi.org/10.1016/s0169-409x(00)00129-0.
40. Verber D.F., Johnson S.R., Cheng H.Y., Smith B.R., Ward K.W. and Kopple K.D. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem*. 2002; 45:2615-2623.
https://doi.org/10.1021/jm020017n.
41. Van de waterbeemd H., Carter R.E., Grassy G., Kubinyi H., Martins Y.C., Tute M.S., et al. Glossary of terms used in computational drug design (IUPAC Recommendations 1997). *Pure Appl. Chem*. 1997; 69:1137-1152.
DOI: https://doi.org/10.1351/pac199769051137.
42. Makhlouf J., Louis H., Benjamin I., Ukwenya E., Valkonen A. & Smirani W. Single crystal investigations, spectral analysis, DFT studies, antioxidants, and molecular docking investigations of novel hexaisothiocyanato chromate complex. *J Mol Struct.* 2023; 1272:134223.
DOI: https://doi.org/10.1016/j.molstruc.2022.134223.
43. Ibezim A., Onuku R., Ottih C., Ezeonu I., Onoabedje E. A., Ramanathan K. and Nwodo N. New sulphonamide-peptide hybrid molecules as potential PBP 2a ligands and methicillin resistant Staphylococcus aureus actives. *J Biomol Struct Dyn,* 2022; 41:6684-6694.
DOI: https://doi.org/10.1080/07391102.2022.2111359
44. Egbujor M.C. Synthesis and biological evaluation of proline derived sulphonamides. *J Res Pharm Sci.* 2023; 9:19-26.
45. Onoabedje E.A., Ibezim A., Okoro U.C. and Batra S. New sulphonamide pyrolidine carboxamide derivatives: Synthesis, molecular docking, antiplasmodial and antioxidant activities. *PLos one.* 2021; 16:e0243305 https://doi.org/10.1371/journal.pone.0243305

46. Ibezim A., Onoabedje E. A., Adaka I. C., Omeje K. and Onoabedje U. S. Carboxamides bearing sulfonamide functionality as potential novel phospholipase A_2 inhibitors. *Chemistryselect*. 2020; 5:14416–14421. DOI: https://doi.org/10.1002/slct.202003784
47. Onoabedje E. A., Ibezim A., Okoro U.C. and Batra S. Synthesis, molecular docking, antiplasmodial and antioxidant activities of new sulfonamido-pepetide derivatives. *Heliyon.* 2020; 6:e04958. DOI: https://doi.org/10.1016/j.heliyon.2020.e04958.
48. Egbujor M.C. Sulfonamide Derivatives: Recent Compounds with Potent Anti- Alzheimer's Disease Activity, *Cent Nerv Syst Agents Med Chem*. 2024; 24:82-104.
DOI:https://dx.doi.org/10.2174/0118715249278489231128042135
49. Maslov O., Komisarenko M., Kolisnyk S. and Derymedvid L. Evaluation of Anti-Inflammatory, Antioxidant Activities and Molecular Docking Analysis of Rubus idaeus Leaf Extract. *Jordan J Pharm Sci*, 2024; *17*(1):105–122.
https://doi.org/10.35516/jjps.v17i1.1808

تصميم، تخليق، ربط جزيئي وتقييم بيولوجي لمشتقات جديدة من السلفامويل بنتاميدات المستخلصة من الليوسين كعوامل مضادة للميكروبات ومضادة للأكسدة

ميلفورد سي. إجبوجور*[1]، فيفيان، آي. أوكونكوا[2]، أوغوما سي. أونيجي[3]، تشيغبوندو إن .إيميروا[4]، أوبونا سي. نكوزينا[5]، بيوس آي. إغواتو[6]، إيفاني سي. أماسيا تو[7]، أليسا سي .أونييمزيري[8]، وأوتشيشوكوو سي. أوكورو[9]

[1] قسم الكيمياء، الجامعة الفيدرالية أوتوكي، ولاية بايلسا، نيجيريا

[2] قسم تكنولوجيا مختبرات العلوم، جامعة نيجيريا، نسوكّا، نيجيريا .

[3] قسم الكيمياء البحتة والصناعية، جامعة نامدي أزيكيوي، أواك، نيجيريا

[4] قسم العلوم الكيميائية، جامعة ريما نيجيريا، أبا، ولاية أبيا، نيجيريا

[5] قسم الهندسة الكيميائية، الجامعة الفيدرالية للتكنولوجيا، أويري، نيجيريا

[6] قسم علم الأحياء الدقيقة، جامعة النهضة، أجباوك، ولاية إنوجو .

[7] قسم الكيمياء الحيوية، جامعة النهضة، أجباوك، ولاية إنوجو .

[8] قسم الكيمياء، الجامعة الفيدرالية للتكنولوجيا، أويري، نيجيريا .

[9] قسم الكيمياء البحتة والصناعية، جامعة نيجيريا، نسوكّا، نيجيريا

ملخص

غالبية الأمراض التي تتوسطها العوامل الميكروبية والإجهاد التأكسدي مقلقة للغاية. تتجلى الحاجة إلى تطوير أدوية جديدة من خلال حقيقة أن مقاومة المضادات الميكروبية في تزايد وأن العديد من الأدوية المضادة للأكسدة الحالية توفر تخفيفًا عرضيًا ضئيلًا فقط. كان الهدف من هذا العمل هو تخليق البنتاميدات السلفامويلية المشتقة من الليوسين ذات الأنشطة المضادة للأكسدة والمضادة للميكروبات. تم تخليق أميدات البنتانامايد السلفامويل القائمة على الليوسين الجديدة وتم استخدام التحليل العنصري، و H-NMR1، و C-NMR13، و FTIRلتوضيح هياكلها. خضعوا لتحقيقات ربط جزيئي بالإضافة إلى تحليلات النشاط المضاد للأكسدة والمضاد للميكروبات في المختبر. المركب 5 a(0.60 جم/مل) كان المركب الأكثر نشاطًا ضد Pseudomonas aeroginosa، بينما كان المركب 5 f(0.30−0.40 ملغ/مل) هو الأكثر فعالية كمضاد للبكتيريا ضد E. Coli و S. typhi و S. aureus و B. subtilis. المركبات ذات أفضل نشاط مضاد للفطريات ضد C. albican و A. niger، على التوالي، كانت 5 0.80) gملغ/مل (و 5 e(0.50 ملغ/مل). في تقييم النشاط المضاد للأكسدة في المختبر، أظهرت المركبات (1.174µg/ml) 5g و5 (1.172µg/ml) hنشاطًا مضادًا للأكسدة مشابهًا لحمض الأسكوربيك (IC50 1.001µg/ml).بالإضافة إلى ذلك، فإن معظم المركبات المستهدفة تتمتع بقدرات قوية نسبيًا مضادة للبكتيريا والفطريات ومضادة للأكسدة، وفقًا لدراسة الربط الجزيئي. نظرًا لأن كل مركب مستهدف امتثل لقواعد ليبينسكي الخمسة، فمن المحتمل أن يتم استخدامه كمرشحين علاجيين لعلاج الأمراض المرتبطة بالإجهاد التأكسدي والعدوى الميكروبية.

الكلمات الدالة: البنتاميدات؛ الليوسين؛ السلفونااميدات؛ مضاد للميكروبات؛ مضاد للأكسدة؛ التخليق.

---

* ***المؤلف المراسل: ميلفورد سي. إجبوجور***

egbujormc@fuotuoke.edu.ng

تاريخ استلام البحث 2024/03/08 وتاريخ قبوله للنشر 2024/05/18.

Antioxidant Activity, Phytochemical Screening, and LC/MS-MS Characterization of Polyphenol Content of Jordanian Habitat of *Pennisetum Setaceum* Aqueous Leaf Extract

Lidia K. Al-Halaseh[1*], Reem Issa[2], Rana Said[3], Rawan Al-suhaimat[1]

[1]Department of Pharmaceutical Chemistry, Faulty of Pharmacy, Mutah University, Al-Karak, Jordan
[2]Department of basic pharmaceutical sciences, Faculty of Pharmacy, Middle East University, Amman, Jordan
[3]Pharmacological and Diagnostic Research Centre, Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, Jordan

ABSTRACT

Background: *Pennisetum setaceum* is an easy-grow and highly adaptable plant characterized by ravishing stalks and colorful leaves. Therefore, this species has been utilized as a green solution in preserving and restoring the ecological balance and developing biodiversity. In addition, different medicinal uses of the plant have been investigated. Yet, modest research was performed to explore the antioxidant activity and the phytochemical composition of the plant.

Objectives: The current research aims to evaluate the phytochemical composition and the antioxidant activity for the Jordanian habitat of *P. setaceum*.

Methods: Aqueous extract of leaves was prepared by maceration. Screening tests for the identification of secondary metabolite content were conducted using standard procedures. The free radical scavenging activity for the extract was determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay and compared with ascorbic acid. The LC-MS/MS analysis was performed focusing on the phenolic content of the extract.

Results: The screening tests revealed the presence of steroids, triterpenoids, alkaloids, tannins, flavonoids, and polyphenols, while saponins were not observed. At a concentration of 4 mg/ml, the free radical scavenging activity for the extract was only 41.32%, compared to 85.54% for ascorbic acid. The LC-MS/MS analysis revealed the presence of eight different phenolic compounds: Succinic acid, protocatechuic aldehyde, 2,5-dihydroxybenzoic acid, 2,3-trans-3,4-trans-leucocyanidin, apiin, iso-orientin, and apigenin, and 5,6,4'-trihydroxy-7,3'-dimethoxyflavone.

Conclusion: The presence of a limited number of phenolic compounds in the *P. setaceum* extract may explain its weak antioxidant activity. Further research is required to identify other (non-phenolic) secondary metabolites content, which would enhances our understanding of the roles this plant species play in agricultural, ecological, or medical applications.

Keywords: *P. setaceum*; Chromatography; Mass Spectroscopy; Secondary metabolites; Antioxidants; Phytochemical screening.

INTRODUCTION

The genus *Pennisetum* (Poaceae) has verified diverse genetic inheritance characteristics according to plant sources and habitats such as China and the United States.[1] Genetic analysis, for identification, was conducted according to simple sequence repeats (SSRs) as DNA markers, and by identifying polymorphic markers.[2,3] A recent study revealed a wide genetic diversity among *Pennisetum* species in ornamental sources.[4]

The species *Pennisetum setaceum* is highly reputed as

***Corresponding author: Lidia Kamal Al-Halaseh**
drhalaseh@mutah.edu.jo
Received: 27/02/2024 Accepted: 28/05/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2442

© 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

an ornamental garden plant,[3] due to its ravishing stalks and colorful leaves.[4] In addition to its aesthetic function, *P. setaceum* has many eco-environmental potential roles; the crop residue has a promising role in adsorbing heavy metal ions in particular, chromium ion from water resources by a non-expensive, efficient absorbent composite based on *P. setaceum* and chitosan. Thus, the plant could be incorporated into a feasible waste management strategy.[5] Besides, a recent study confirmed the efficiency of *P. setaceum* in treating sanitary sewage effluent from pharmaceutical contaminants including caffeine, endocrine disruptors, and other pharmaceuticals.[6] Recent research studied the behavior of some ornamental plants, including *P. setaceum*, and concluded their importance as a green solution in preserving and restoring the ecological balance and developing biodiversity.[7,8]

In regard to the use of *P. setaceum* as a medicinal plant and exploring its biological activities, modest data have been collected from research projects performed to justify the use of the plant for medicinal purposes. Ethnopharmacological and scientific studies revealed that the plant was used in managing urogenital pain in Sudan, lowering blood sugar level in Jordan, and as a slimming agent.[9,10,11]

A review article published by Ojo et al.,[12] mentioned that only a few species' chemical compositions and metabolites have been investigated in the genus *Pennisetum*, with varied health related activities. These secondary metabolites include varied fatty acids, anthocyanin, steroids, triterpenoids, alkaloids, tannins, flavonoids, polyphenols, and saponins. The secondary metabolites content of the plant of our interest are not fully-investigated yet, except for anthocyanin content and biosynthesis .[13,14]

It is well known that oxidative stress has been linked to a variety of degenerative diseases. Therefore, there is a greater demand for the use of antioxidants agents such as phenolic compounds in addition to other micronutrients.[12, 15, 16] The, current study aims to screen the antioxidant activity (via DPPH assay), and the secondary metabolites content (via general phytochemical screening and LC/MS-MS characterization of polyphenols) of the Jordanian habitat of *P. setaceum* aqueous leaf extract. Findings would shed light on the potential biological activities, as well as the phytochemical composition of the plant, with special focus on the antioxidant and polyphenols components. To our knowledge, this is the first study investigating the antioxidant activity, phytochemical composition, and phenolic content of this plant species.

MATERIALS AND METHODS

Plant material

Fresh leaves were collected from widely grown plants in South Jordan. The plant material was identified by the Royal Society for the Conservation of Nature (RSCN), Amman, Jordan. A voucher sample was deposited in the RSCN herbarium with the reference number RS/10/1/518.

According to Al-Halaseh et al.[11], the leaves were dried under shade before reducing in size. For extraction, each 250 g of dried leaves was macerated with 100 ml distilled water with continuous stirring using mmemert shaker waterbath® at 25°C for 16 h. This process was repeated twice, then the suspension was filtered and concentrated until a fine powder was obtained using Benchtop Manifold Freeze Dryer from Millrock Technology®.

Preliminary phytochemical analysis

Phytochemical screening tests were performed to identify the major secondary metabolite content. Polysteroids and triterpenoids content were checked according to Leiberman–Buchard and Salkowski assays, respectively.[16] Saponin content was checked by the frothing test, and ferric chloride solution (1%) was used to identify the soluble polyphenol and tannin content. The Kumar method was used to identify the presence of flavonoids.[17]

Antioxidant activity for P. setaceum aqueous leaf extract

A colorimetric assay based on using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent was used to evaluate the

radical scavenger activity of *P. setaceum* aqueous leaf extract following published data.[18] For the reaction reagent, 0.4 g of DPPH was dissolved in 100 ml methanol. The reaction was performed by mixing 0.1 g of plant extract with 10 ml methanol. 1 ml of the plant extract solution was mixed with 3 ml of DPPH and completed to a final volume of 10 ml using methanol, then allowed to stand in darkness for 30 minutes. The calibration curve was done using ascorbic acid at serial concetrations as a standard antioxidant (Sigma Aldrich, Germany).

The effective concentration required for scavenging of DPPH free radicals (inhibition%) was calculated using the plotted graph of scavenging activity verses the extract concetrations and compared to ascrbic acid. The percentage inhibition (I%) was computed for each sample after measuring the absorbance at λ_{max}=517 nm using Equation 1.

$$\%\ inhibition = [\frac{A_{control} - A_{sample}}{A_{control}}] \times 100$$

Where: $A_{control}$ = absorbance of the control sample, and A_{sample} = absorbance of the sample

Equation 1. DPPH inhibition percentage equation

LC/MS-MS Methodology

Instrumentation and MS parameters

A Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker Dalotonik Elute UPLC system (Bremen, Germany) was used for screening the compounds of interest. This instrument was operated using the Ion Source Apollo II ion funnel electrospray source. The capillary voltage was 2500 V, the nebulizer gas was 2 bar, the dry gas (nitrogen) flow rate was 8 L/min and the dry temperature was 200 ºC. The mass accuracy was < 1 ppm; the mass resolution was SR (Full Sensitivity Resolution) and the TOF repetition rate was up to 20 kHz. UHPLC was coupled to a Bruker impact II QTOFMS.

Chromatographic separation was performed using Bruker Solo 2.0_C-18 UHPLC column (100 mm x 2.1 mm x 2.0 µm) at a flow rate of 0.51 ml/min and a column temperature of 40 ℃. The mobile phase consists of (A) water with 0.05% formic acid and (B) acetonitrile. Gradient mode was applied according to the following: 0 – 27 min linear gradient from 5% - 80% B; 27-29 min 95% B; 29.1 min 5% B. The total analysis time was 35 min with an injection volume of 3 µl.

A previously developed integrated library of natural compounds was used for identification of the phenolic compounds based on the RT and m/z with high resolution. Samples tock solutions were prepared by dissolving an appropriate amount of the plant extract in 2 ml dimethyl sulfoxide-DMSO (analytical grade), followed by dilution with acetonitrile up to 50 ml and centrifugation at 4000 rpm, for 2 min.

This study also includes a quantitative estimates of the percentage (%) relative content of phenolic components, using the method described by Mohammed et al. [19] The area under the peak for each identified phenolic component was converted into peak area (%), showing the occurrence levels of the identified compounds, that were calculated relative to the total area of all the peaks observed in the LC-chromatogram.

RESULTS

Extraction yield

The percentage of extractable compounds using water and maceration extraction method varied from 3.9% to 4.1% (w/w).

Qualitative screening of secondary metabolites

According to the obtained results, *P. setaceum* aqueous leaf extract showed to contain steroids, triterpenoids, tannins, flavonoids, and polyphenols, while the saponins test showed negative result.

Antioxidant activity (DPPH Assay) for P. setaceum aqueous leaf extract

The antioxidant efficiency, expressed by the free radical inhibition activity, was calculated for serial concentrations of the plant extract, and compared to the standard ascorbic acid (Figure 1). Findings revealed that the plant extract

possesses weak antioxidant activity compared to the standard ascorbic acid. For example, at the higher tested concentration (4.0 mg/ml), the percentage inhibition of the extract was found to be 41.32% compared to 85.54% for the ascorbic acid at the same tested concentration.

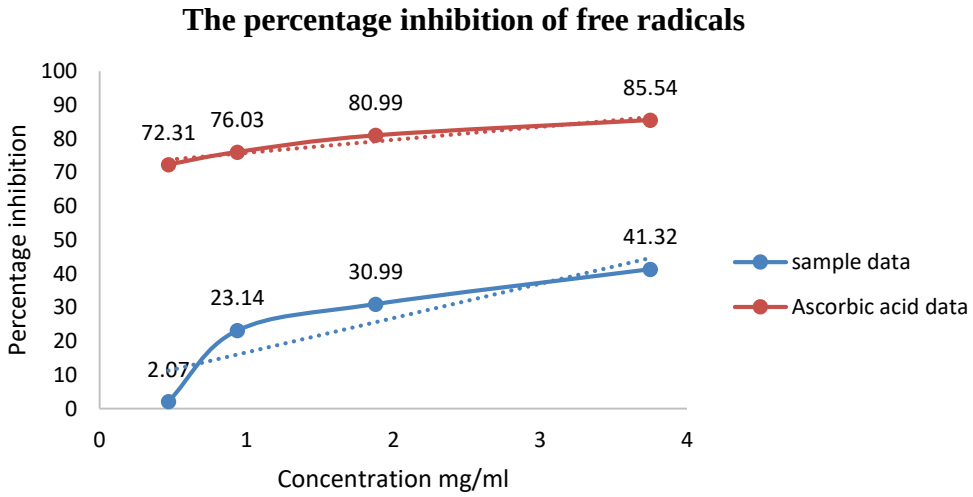


Figure 1: The %inhibition of free radical activity for serial concentrations of plant extracts and ascorbic acid, using the DPPH assay method.

Identification of phenols using LC/MS-MS analysis

Eight different phenolic components have been detected using the LC/MS-MS analysis, and the integrated natural compounds library. The detected compounds are listed in Table 1. Estimation for the individual percentage content of the detected compound showes that succinic acid to be the most abendant compond (48%). Other componds were abendant in the range of (5-9%) relative to the total detected componds.

Table 1: Components detected in *P. setaceum* aqueous leaves extract based on retention time (RT) and Mass (m/z) using LC-MS/MS analysis.

RT [min]	m/z meas.	M meas.	Name	Molecular Formula	Peak area (%)*
0.98	117.0194	118.0267	Succinic acid	$C_4H_6O_4$	48%
1.23	137.0246	138.0318	Protocatechuic aldehyde	$C_7H_6O_3$	5%
1.75	153.0195	154.0268	2,5-Dihydroxybenzoic acid	$C_7H_6O_4$	8%
3.59	305.0699	306.0772	2,3-Trans-3,4-trans-Leucocyanidin	$C_{15}H_{14}O_7$	6%
4.87	563.1414	564.1486	Apiin	$C_{26}H_{28}O_{14}$	9%
4.9	447.0928	448.1001	ISO-Orientin	$C_{21}H_{20}O_{11}$	9%
5.67	607.1303	608.1376	Apigenin	$C_{27}H_{28}O_{16}$	7%
10.3	329.0671	330.0743	5,6,4'-Trihydroxy-7,3'-dimethoxyflavone	$C_{17}H_{14}O_7$	8%

* Relative % (percentages) of the occurrence levels of the identified compounds were calculated in relative to the total area of all the peaks detected in the LC-chromatogram.

Figure 2 shows the total ion chromatogram for all compounds detected in *P. setaceum aqueous* leaves extract. The LC-chromatograms showing peaks and retention time of each compound detected in the extract are shown in Figure 3. Figure 4 shows the Mass spectra (m/z) and fragments for each compound detected in the extract.

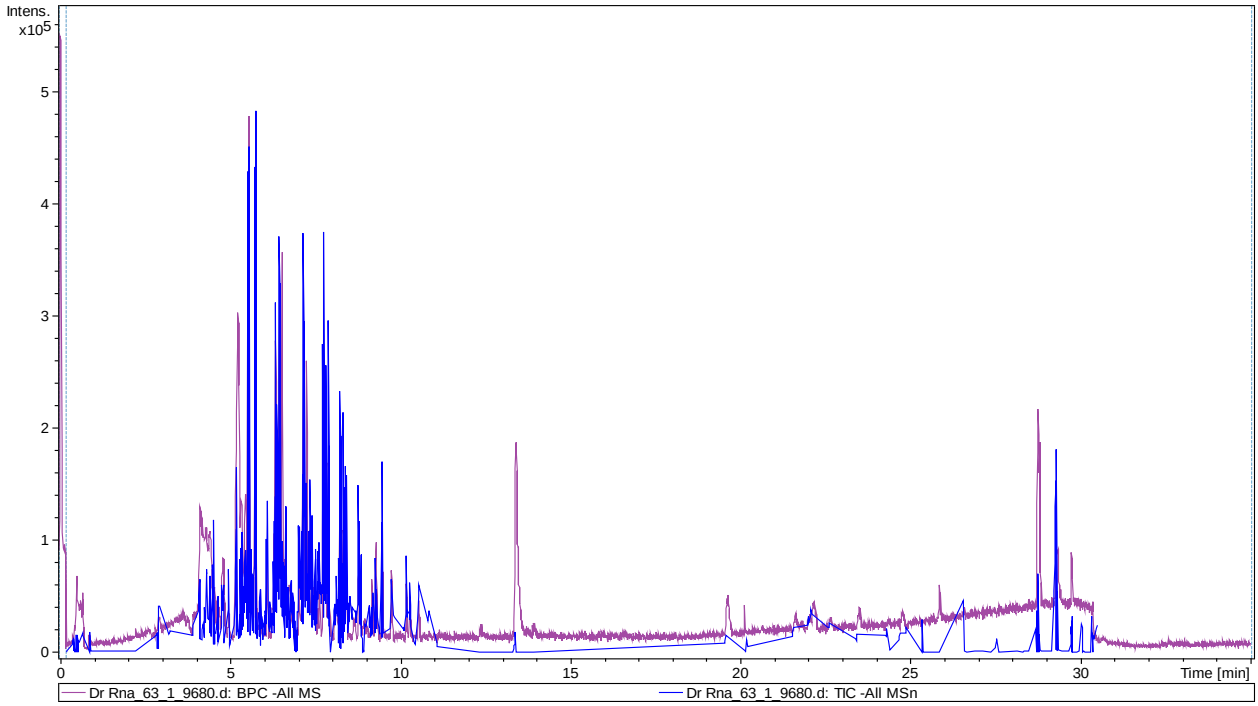


Figure 2. Total ion chromatogram for all compounds detected in *P. setaceum aqueous* leaves extract.

DISCUSSION

Fountain grass, *P. setaceum*, is highly reputed as an ornamental plant, in addition to its contribution to the ecosystem.[4,9] However, the medicinal activity has not been thoroughly investigated, with limited studies are available on this species.[10, 11, 12] Mostly, the biological activity of a plant extract is attributed to the secondary metabolite content and the antioxidant activity[19]. Therefore, the current study aimed to screen the content of secondary metabolite, phenolic composition, and the antioxidant activity of the *P. setaceum* extract, and to compare it with the content of similar groups among the other related plant species.

In this study, the obtained phytochemical screening results revealed the presence of different bioactive metabolites in the aqueous extract of the Jordanian fountain grass leaves. These include water-soluble polyphenols, flavonoids, polysteroids, triterpenoids, and tannins. The test failed to detect the presence of saponins. These findings were expected, as previous studies investigated the related plant species; *P. purpureum* phytochemical composition revealed the presence of tannins (67.9%), alkaloids (lunamarine 26.21%), phenols (1.16%), and flavonoids (rutin 2.10%, anthocyanin 0.06%, catechin 2.49%, and kampferol 0.09%). Similarly, seven phenolic compounds (Trans-cinnamic, protocatechic, hydroxybenzoic acids, Gentisic, gallic, caffeic, and p-Coumaric) and two flavonoids (quercetin and catechin) were found in the species *P. glaucum* oil extract.[12]

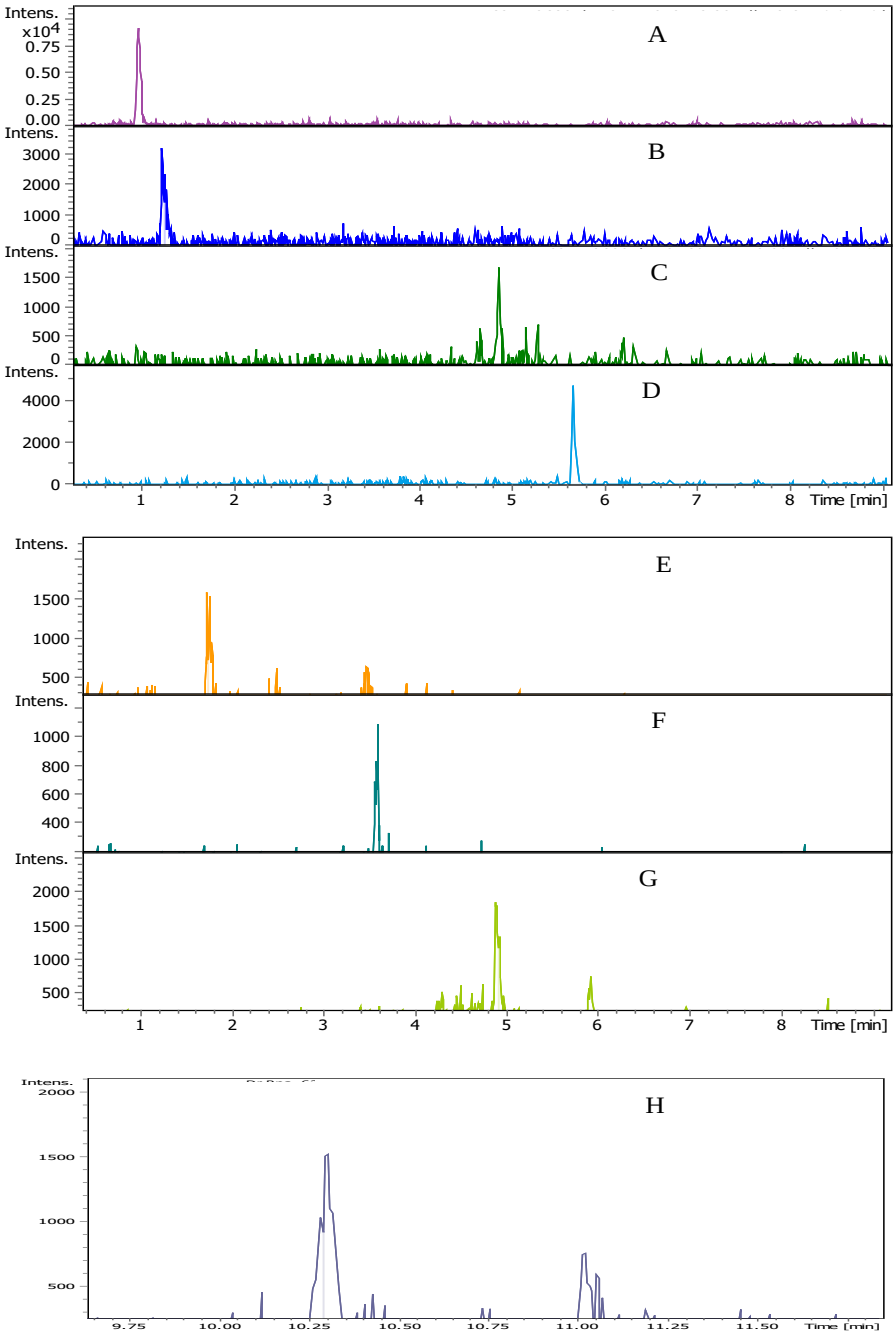


Figure 3. The LC-chromatograms showing peaks and retention time of each compound detected in *P. setaceum aqueous aqueous* leaves extract; (A) Succinic acid, (B) Protocatechuic aldehyde, (C) Apiin, (D) Apigenin, (E) 2,5-Dihydroxybenzoic acid, (F) 2,3-Trans-3,4-trans-Leucocyanidin, (G) ISO-Orientin, (H) 5,6,4'-Trihydroxy-7,3'-dimethoxyflavone.

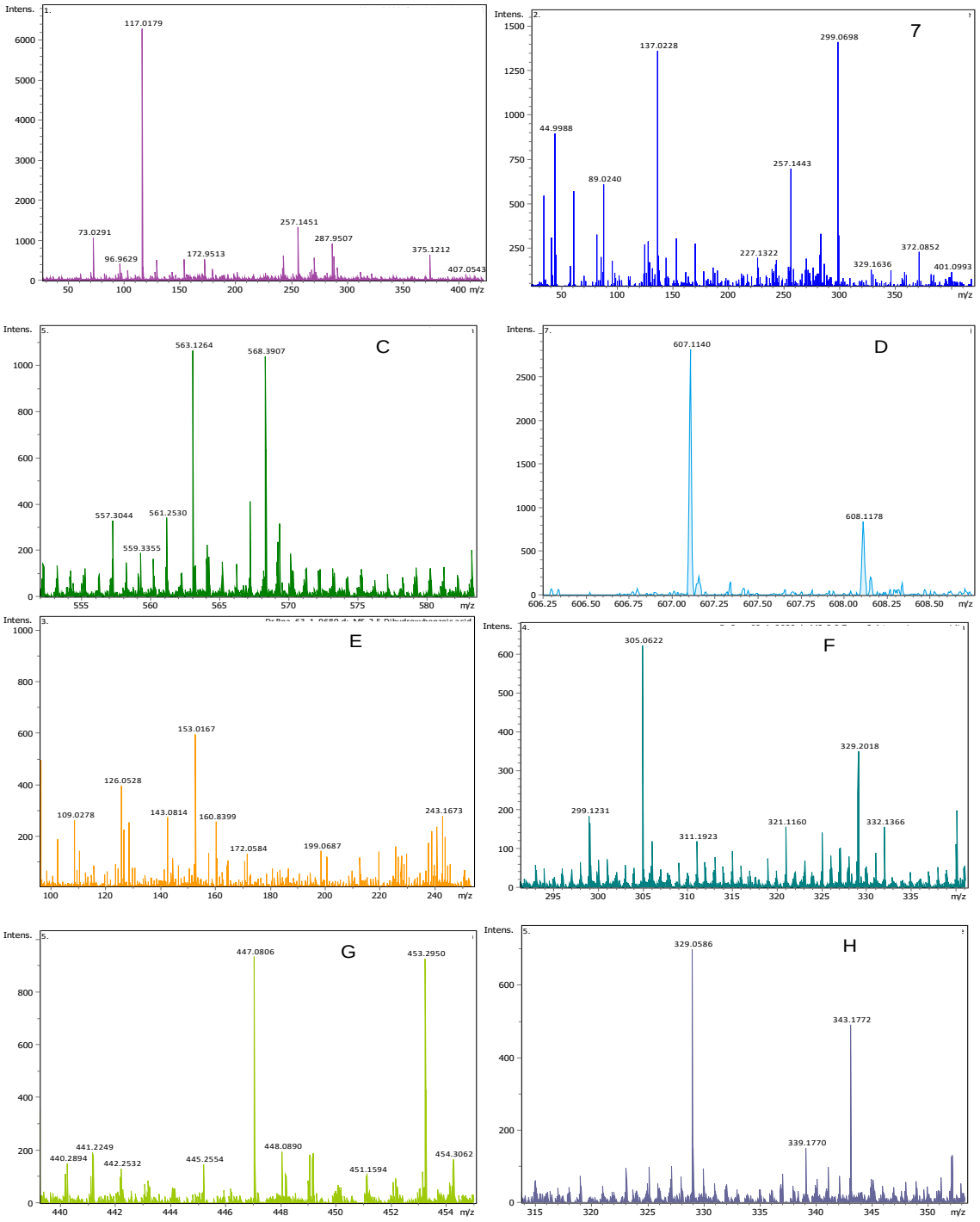


Figure 4. MS/MS spectra showing m/z and fragments of each compound detected in *P. setaceum aqueous* leaves extract; (A) Succinic acid, (B) Protocatechuic aldehyde, (C) Apiin, (D) Apigenin, (E) 2,5-Dihydroxybenzoic acid, (F) 2,3-Trans-3,4-trans-Leucocyanidin, (G) ISO-Orientin, (H) 5,6,4'-Trihydroxy-7,3'-dimethoxyflavone.

The free radical scavenging activity of the tested plant extract revealed a weak antioxidant activity compared to the reference ascorbic acid.[18] These results were explained by the findings of the phenolic compounds analysis using the LC/MS-MS, showed the presence of only 8 compounds of these biologically active phenolic compounds, which are widely known for contributing to the antioxidant activity for plants extracts. Similarly, the antioxidant tests using the DPPH or others examining the extract of *P. glaucum* had shown to possess low antioxidant activities, which was influenced by the various ways in which the plant was processed.[12] These findings were explained as when the plant was being exposed to increasingly high temperatures, the flavonoid content of the plant decreases, resulted in a reduction in the antioxidant activity .

In this study, investigating the polyphenol content of the plant extracts, eight metabolites with well-known biological and medicinal activities were identified using the LC/MS-MS in the aqueous extract. Of those the most abundant was succinic acid, is a dicarboxylic acid with a conventional use as an intermediate for variable pharmaceuticals and other consumer products.[20, 21 , 22]

The phenolic acid, protocatechuic acid, has a well-known antioxidant activity and was found to treat and/or prevent activities against a multiplicity of disorders, showed activity against bacterial, fungal, and viral infections. Furthermore, the hypotensive, hypolipidemic, and bronchodilation activities were elucidated by several *in vitro* studies, in addition to antispasmodic properties.[23]

A synthetic water-soluble formula of 2,5-hydroxybenzoic acid (2,5-DHBA) and gelatin has a promising antiviral activity by inhibiting the adsorption of alpha-herpesviruses to cells.[24] The metabolite 2,3-Trans-3,4-trans-Leucocyanidin is an antioxidant and serves as a precursor to proanthocyanidin biosynthesis.[25] Apiin is a widely distributed natural flavonoid with reported anti-inflammatory and immunomodulatory activities.[26] A potential antidiabetic activity for iso-orientin has been reported in *silico* and animal model studies.[27] A health-promoting effect of apigenin was reported after several *in vivo* studies. It has beneficial effects on hypoglycemia, memory enhancement, sleep aid, and anticancer activities.[28] Apigenin was reported as a potential chemotherapeutic agent.[29] In addition, its derivative "genistein" is an isoflavone, isolated previously from soybean, has a chemopreventive effect at low therapeutic doses, with a pharmacological effect at high administered oses.[30]

The eighth detected metabolite, 5,6,4'-Trihydroxy-7,3'-dimethoxyflavone is not an exception. A pharmacological study revealed its potent antioxidant and anti-inflammatory activities. The metabolite reduces the production of nitric acid and cytokine and interferes with nuclear factor-κB translocation and mitogen-activated protein kinase pathways.[31] The revealed antioxidant activity of the current extract matches the expectations, where multiple plants showed free radical scavenger activities in variable efficacies. [32-34]

CONCLUSION

The LC-MS/MS analysis of *P. setaceum* aqueous leaves extract reveals the presence of a limited number of phenolic compounds. These findings would explain the weak antioxidant activity observed for the extract. Further research is required to identify the other (non-phenolic) secondary metabolites, observed using the screening tests, which would enhance our understanding of potential uses of this plant species for agricultural, ecological, or medical applications.

Author contribution: All authors contributed to the research works and approved the submitted manuscript.

Conflict of interest: All authors declare no conflict of interest.

Data availability: Data is available from corresponding authors upon reasonable requests.

REFERENCES

1. Zhou S., Wang C., Yin G., Zhang Y., Shen X, Pennerman K.K., Zhang J., Yan H., Zhang C., Zhang X. and Ren S. Phylogenetics and diversity analysis of Pennisetum species using Hemarthria EST-SSR markers. *Grassland science*. 2019; 65(1):13-22.
2. Wang C., Yan H., Li J., Zhou S., Liu T., Zhang X. and Huang L. Genome survey sequencing of purple elephant grass (Pennisetum purpureum Schum 'Zise') and identification of its SSR markers. *Molecular Breeding*. 2018; 38:1-0.
3. Zhang Y., Yuan X., Teng W., Chen C. and Wu J. Identification and phylogenetic classification of Pennisetum (Poaceae) ornamental grasses based on ssr locus polymorphisms. *Plant Molecular Biology Reporter*. 2016; 34:1181-92.
4. Guo Y., Liu L., Yue Y., Fan X., Teng W., Zhang H., Gao K., Guan J., Chang Z. and Teng K. Development of SSR Markers Based on Transcriptome Sequencing and Verification of Their Conservation across Species of Ornamental Pennisetum Rich. (Poaceae). *Agronomy*. 2022; 12(7):1683.
5. Nguyen M.L. Potential Elimination of Chromium (VI) by Chemically Biochar Derived from Pennisetum Setaceum. *Journal of Technical Education Science*. 2022; 28(73):74-9.
6. Melo A.F., Oliveira J.F., Fia F.R., Fia R., Matos M.P. and Sanson A.L. Microcontaminants Removal in Constructed Wetlands with Different Baffle Arrangements and Cultivated with Pennisetum setaceum. *Water, Air, & Soil Pollution*. 2022; 233(8):322.
7. Rowe H.I., Sprague T.A. and Staker P. Comparing common fountain grass removal techniques: cost efficacy and response of native plant community. *Biological Invasions*. 2022; 24(12):3817-30.
8. Oprea M.I., Daniela Giosanu M. Decorative herbs, a green solution for urban arrangements. *Current Trends in Natural Sciences*. 2022; 11(21):356-64.
9. Madani I., Tahir Y.F. and Nour S.M. Ethnobotanical Study of Medicinal Plants Used by El Kababish Tribe in Ga'ab El Lagia Oasis, West Dongola (Sudan). *Nyame Akuma Bulletin*. 2014; 82:91-9.
10. Al-Halaseh L.K., Al-Jawabri N.A., Al-Btoush H., Al-Suhaimat R., Majali S., Hajleh M.N., Ali A.S. and Ahmed M A. In vivo investigation of the potential hypoglycemic activity of Pennisetum setaceum: Justification of the traditional use among Jordanians. *Research Journal of Pharmacy and Technology*. 2022;15(7):3185-9.
11. Al-Halaseh L.K., Hajleh M.N. and Al-Samydai A.M. Weight reduction in response to unauthorized treatment in a poorly controlled obese woman. *Biomedicine*. 2022; 42(2):410-2.
12. Ojo O.A., Ojo A., Barnabas M., Iyobhebhe M., Elebiyo T.C., Evbuomwan I.O. and Oloyede O.I. Phytochemical properties and pharmacological activities of the genus Pennisetum: A review. *Scientific African*. 2022; 16, e01132.
13. Beckwith A.G., Zhang Y., Seeram N.P., Cameron A.C. and Nair M.G. Relationship of light quantity and anthocyanin production in Pennisetum setaceum cvs. Rubrum and Red Riding Hood. *Journal of agricultural and food chemistry*. 2004; 52(3):456-61.
14. Liu L., Teng K., Fan X., Han C., Zhang H., Wu J. and Chang Z. Combination analysis of single-molecule long-read and Illumina sequencing provides insights into the anthocyanin accumulation mechanism in an ornamental grass, Pennisetum setaceum cv. Rubrum. *Plant Molecular Biology*. 2022; 109(1-2):159-75.
15. Yang X., Yang N., Zhang Q., Pei Z., Chang M., Zhou H., Ge Y., Yang Q. and Li G. Anthocyanin biosynthesis associated with natural variation in autumn leaf coloration in Quercus aliena accessions. *International Journal of Molecular Sciences*. 2022; 23(20):12179.
16. Cock I.E. and Kukkonen L. An examination of the medicinal potential of Scaevola spinescens: Toxicity, antibacterial, and antiviral activities. *Pharmacognosy Research*. 2011; 3(2):85.

17. Al-Halaseh L.K., Sweiss M.A., Issa R.A., AlKassasbeh R., Abbas M.A., Al-Jawabri N.A., Hajleh M.N. and Al-Samydai A.M. Nephroprotective Activity of Green Microalgae, Chlorella sorokiniana Isolated from Jordanian Water. *Journal of Pure & Applied Microbiology*. 2022; 16(4).
18. Lali M.A., Issa R.A., Al-Halaseh L.K., Al-Suhaimat R. and Alrawashdeh R. Reduction of reproductive toxicity in murine sperm model using Moringa peregrina leaves extracts. *Journal of Applied Pharmaceutical Science*. 2023; 13(11):050-6.
19. Mohammed H.A., Ali H.M., Qureshi K.A., Alsharidah M., Kandil Y.I., Said, R. and Khan R.A. Comparative phytochemical profile and biological activity of four major medicinal halophytes from Qassim flora. *Plants*. 2021; 10(10), 2208.
20. Saxena R.K., Saran S., Isar J. and Kaushik R. Production and applications of succinic acid. *In: Current developments in biotechnology and bioengineering*. 2017; 601-630.
21. Zhang Q.C., Zhao Y. and Bian H.M. Antiplatelet activity of a novel formula composed of malic acid, succinic acid and citric acid from Cornus officinalis fruit. *Phytotherapy Research*. 2013; 27(12):1894-6.
22. Nidamarthi H.K., Choudhury M. and Velmurugan D. Understanding the binding mechanism of succinic acid against phospholipase A2 from bee venom. *Journal of Biochemical and Molecular Toxicology*. 2021; 35(4): e22715.
23. Kakkar S. and Bais S. A review on protocatechuic acid and its pharmacological potential. *International Scholarly Research Notices*. 2014; 2014:1-9.
24. Lisov A., Vrublevskaya V., Lisova Z., Leontievsky A. and Morenkov O. A 2, 5-Dihydroxybenzoic acid–gelatin conjugate: the synthesis, antiviral activity and mechanism of antiviral action against two alphaherpesviruses. *Viruses*. 2015; 7(10):5343-60.
25. Jun J.H., Lu N., Docampo-Palacios M., Wang X. and Dixon R.A. Dual activity of anthocyanidin reductase supports the dominant plant proanthocyanidin extension unit pathway. *Science Advances.* 2021; 7(20):1-11.
26. Mencherini T., Cau A., Bianco G., Loggia R.D., Aquino R.P. and Autore G. An extract of Apium graveolens var. dulce leaves: Structure of the major constituent, apiin, and its anti-inflammatory properties. *Journal of pharmacy and pharmacology*. 2007; 59(6):891-7.
27. Malik A., Jamil U., Butt T.T., Waquar S., Gan S.H., Shafique H. and Jafar T.H. In silico and in vitro studies of lupeol and iso-orientin as potential antidiabetic agents in a rat model. *Drug design, development and therapy*. 2019; 1501-13.
28. Salehi B., Venditti A., Sharifi-Rad M., Kręgiel D., Sharifi-Rad J., Durazzo A., Lucarini M., Santini A., Souto E.B., Novellino E. and Antolak H. The therapeutic potential of apigenin. *International journal of molecular sciences*. 2019; 20(6):1305.
29. Rampogu S., Gajula R.G. and Lee K.W. A comprehensive review on chemotherapeutic potential of galangin. *Biomedicine & Pharmacotherapy*. 2021; 141:111808.
30. Russo M., Russo G.L., Daglia M., Kasi P.D., Ravi S., Nabavi S.F. and Nabavi S.M. Understanding genistein in cancer: The "good" and the "bad" effects: A review. *Food chemistry*. 2016; 196:589-600.
31. Wang S.H., Liang C.H., Liang F.P., Ding Y., Lin S.P., Huang G.J., Lin W.C. and Juang S.H. The inhibitory mechanisms study of 5, 6, 4′-trihydroxy-7, 3′-dimethoxyflavone against the LPS-induced macrophage inflammatory responses through the antioxidant ability. *Molecules*. 2016; 21(2):136.
32. Alkhatib R. Chemical Composition of Essential Oils Total Phenols and Antioxidant Activity of Achillea fragrantissima and A. santolina Grown in Syria. *Jordan Journal of Pharmaceutical Sciences.* 2024; 17(3):594-602.
33. Puspitasari E., Triatmoko B., Dianasari D., Muslichah S. and Nugraha A.S. Assessment of Extraction Methods Effects on the Biological Activities (Antioxidant and Antiamylase) and Chemistry (Total Phenolics and Flavonoids) of Guazuma ulmifolia Leaves. *Jordan Journal of Pharmaceutical Sciences*. 2024; 17(1).
34. Naskar A., Dasgupta A. and Acharya K. Antioxidant and cytotoxic activity of Lentinus fasciatus. *Jordan Journal of Pharmaceutical Sciences*. 2023; 25;16(1):72-81.

نشاط مضادات الأكسدة والفحص الكيميائي النباتي وتوصيف LC / MS-MS لمحتوى البوليفينول في الموائل الأردنية لمستخلص الأوراق المائية *Pennisetum setaceum*

ليديا كمال الهلسه[1] *، ريم عيسى[2]، رنا سعيد[3]، روان السحيمات[1]

[1] قسم الكيمياء الصيدلانية، كلية الصيدلة، جامعة مؤته، الأردن

[2] قسم الصيدلة الاساسية، كلية الصيدلة، جامعة الشرق الاوسط، الأردن

[3] مركز الابحاث الدوائية، كلية الصيدلة، جامعة عمان الاهلية، الأردن

ملخص

الخلفية: *Pennisetum setaceum* هو نبات سهل النمو وقابل للتكيف بدرجة كبيرة ويتميز بالسيقان الجميلة والأوراق الملونة. لذلك،تم استخدام هذا النوع كوسيلة خضراء في الحفاظ على التوازن البيئي واستعادته وتطوير التنوع البيولوجي. بالإضافة إلى ذلك،تم التحقق من بعض الاستخدامات الطبية المختلفة لهذا النبات. تم سابقا إجراء بعض الابحاث البسيطة لاستكشاف النشاط المضاد للأكسدة والتركيب الكيميائي النباتي لأوراق هذا النبات.

الأهداف: يهدف هذا البحث إلى تقييم التركيب الكيميائي النباتي وفعالية مضادات الأكسدة للموئل الأردني لنبات *P.setaceum*. المنهجية: تم تحضير المستخلص المائي للأوراق عن طريق النقع. أجريت اختبارات تحليلية لتحديد المستقلبات الثانوية النشطة بيولوجيا والناتجة عن الأيض الثانوي باستخدام إجراءات مرجعية. تم تحديد نشاط مضاد الاكسدة للجذور الحرة للمستخلص باستخدام مقايسة DPPH، (2-Diphenyl-1-picrylhydrazyl 2) ومقارنتها بحمض الأسكوربيك. تم إجراء تحليل LC-MS/MS مع التركيز على المحتوى الفينولي للمستخلص.

النتائج: كشفت الاختبارات التحليلية عن وجود للستيرويدات، ترايتيربينويدات، قلويدات، العفص، الفلافونويد، والبوليفينول، في حين لم يلاحظ وجود الصابونينات. عند تركيز 4 ملغ/مل، كان نشاط مضاد الاكسدة للجذور الحرة للمستخلص 41.32٪ فقط، مقارنة ب 85.54٪ لحمض الأسكوربيك. كشف تحليل LC-MS/MS عن وجود ثمانية مركبات فينولية مختلفة:حمض السكسينيك، وألدهيد البروتوكاتيكويك، وحمض 2،5–ثنائي هيدروكسي بنزويك، و2،3–ترانس–4،3–ترانس–ليوكوسيانيدين، وأبين، وإيزو–أورينتين، وأبيجينين، و4،6،5'–ثلاثي هيدروكسي–3،7'–ديميثوكسي فلافون.

الخلاصة: وجود عدد محدود من المركبات الفينولية في مستخلص *P.setaceum* قد يفسر ضعف نشاطه المضاد للأكسدة. هناك حاجة إلى مزيد من البحث لتحديد المستقلبات الثانوية الأخرى (غير الفينولية)، والتي من شأنها أن تعزز فهمنا لدور هذا النوع النباتي في التطبيقات الزراعية أو البيئية أو الطبية.

الكلمات الدالة: كروماتوغرافي، سبكتروسكوبي، مضادات أكسدة، نواتج طبيعية ثانوية، *P.setaceum*، الفحص الكيميائي النباتي.

**** المؤلف المراسل: ليديا كمال الهلسه***

drhalaseh@mutah.edu.jo

تاريخ استلام البحث 2024/02/27 وتاريخ قبوله للنشر 2024/05/28.

Jordan Journal of Pharmaceutical Sciences, Volume 17, No. 4, 2024

Impact of COVID 19 Pandemic on the Mental Health of Diabetic Patients in Jordan: An Online Survey

Ghaith M Al-Taani[1]*, Suhaib M Muflih[2], Rawan Alsharedeh[3], Reema Karasneh[4], Sayer I Al-Azzam[2]

[1] Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy, Yarmouk University, Irbid, Jordan
[2] Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan
[3] Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Yarmouk University, Irbid, Jordan
[4] Department of Basic Medical Sciences, Faculty of Medicine, Yarmouk University, Irbid, Jordan.

ABSTRACT

It is envisioned that the COVID-19 pandemic has influenced the mental health of many individuals, especially those who have diabetes. The present study evaluated the effect of the COVID-19 pandemic on diabetics' mental health. A pretested online questionnaire was developed to assess the concern related to COVID-19, anxiety and depressive symptoms, and resilience to stress of people with diabetes during the COVID-19 pandemic. Data from 371 surveys was collected. The majority (71.2%) of the responders' aged between 41 to 64 years. Chronic disease was present in 69.3% of respondents, while 76.8% indicated overall good health. Most respondents (82.5%) were worried a lot or worried about the economic recession, whilst 53.9% of respondent were worried a lot or worried about frightening media messages. Around 35% of those surveyed were feeling depressed for at least three days a week for the previous two months, and 30.5% were fearful for at least three days a week for the previous two months. Approximately 60% of respondents agreed or strongly agreed that they had difficulty getting through stressful events. The current study revealed elevated rates of fear and depression symptoms, and noticeable difficulties in managing stressful situations associated with the COVID-19 pandemic within diabetes patients. These results highlight the importance of addressing the mental health issues within the chronic diseases patients at the time of crises. Which can be realized via specific interventions at the time of pandemics, which would reflect on the overall mental well-being and ability to adapt in stressful situations.

KEY WORDS: COVID-19; Mental health; Diabetes Mellitus; Jordan

INTRODUCTION

Diabetes is increasing in prevalence in the Middle East and Eastern Mediterranean countries and has been described as an “epidemic” in propagation by the World Health Organization [1, 2]. It is characterized as a progressive chronic disease with potentially severe complications. These characteristics, among others, can evoke various emotions in individuals with diabetes, notably depressive symptoms. Research has shown a higher incidence of depression among diabetic patients compared to non-diabetics [3, 4].

A particularly challenging reality is that depression exacerbates the deterioration of diabetics’ health, quality of life, and overall functionality [5-9]. Co-existing diabetes and depression significantly increase the burden of the disease, adversely affecting social and vocational functioning, increasing functional disability, and heightening the risk of complications and mortality. These outcomes are influenced, in part, by psychological factors

*Corresponding author: Ghaith M Al-Taani
g.altaani@yu.edu.jo
Received: 14/01/2024 Accepted: 13/06/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2234

© 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

and issues related to medication adherence and lifestyle behaviors [3, 6, 7, 10].

During the outbreak of COVID-19, managing the pandemic was challenging due to its unique characteristics, specifically its rapid spread and highly contagious nature, which significantly impacted daily life in both developed and developing countries, including Jordan [11]. To successfully control the epidemiological situation during the outbreak and in the future, it is crucial for the average person in Jordan to adhere to infection control measures. These measures were disseminated to the public through various channels in collaboration with the mass media, government, Ministry of Health, community institutions, private companies, and other stakeholders.

Insights gained from previous outbreaks, such as the SARS outbreak in 2003, revealed that fear and panic could complicate efforts to address public health crises. To avoid irrational fear, it is essential to communicate accurate information about outbreaks and place such fears in the proper perspective [12, 13].

Patients with diabetes and other chronic diseases are considered at higher risk for complications from COVID-19. Research indicates that diabetics are more likely to experience severe medical complications from COVID-19, such as respiratory issues. COVID-19 progresses more rapidly in high blood glucose environments and can trigger diabetes-related complications, such as diabetic ketoacidosis. However, it is important to note that patients with diabetes do not have a higher risk of contracting COVID-19 itself [14].

Given this context—and the extensive media coverage of COVID-19 cases, deaths, quarantine interventions, and public education on protective measures such as face masks and social distancing—there is an increased likelihood that the pandemic may amplify the expression of various emotions, particularly in diabetic patients. This study assessed the impact of the COVID-19 pandemic and quarantine interventions on the mental health of diabetic individuals, focusing on symptoms of fear, anxiety, and depression, as well as associated risk factors.

METHODS

An online survey using Google Forms was created to collect data from diabetics in Jordan. The survey was efficiently distributed through a specific Facebook page, *Diabetes and Life*, which includes Jordanian diabetics who regularly post health-related messages, offer support, and provide helpful resources to patients with diabetes. During the COVID-19 period, similar surveys were conducted by other authors [15-17].

The instrument consisted of 28 items designed to assess the impact of COVID-19 on the mental health of diabetic patients. It included:

- Demographic information: (8 items) such as age, employment status (employed, unemployed, student, retired), area of residence (rural, urban), health insurance status (yes, no), educational level (school, university, postgraduate studies), presence of chronic diseases, and overall health status.

- An adapted version of the validated Worry Domains Questionnaire (WDQ), which measures non-pathological worries [18]. The current level of worry was assessed using an adapted Likert scale with the following responses: *worry a lot*, *worry*, and *not worry at all*. This scale focused on bespoke worry items (10 items) envisioned to arise from the COVID-19 pandemic, such as losing a loved one due to COVID-19, increased pressure on the health system, and restrictions on freedom (e.g., restricted movement).

A summated fear score (ranging from 0 to 20) was calculated for each participant. Responses were weighted as follows: 0 for *don't worry*, 1 for *worry*, and 2 for *worry a lot*. The scores were then summed, with higher scores indicating more severe fear symptoms.

- A set of six items addressing the degree of anxiety and depression over the past two months was selected from the validated Center for Epidemiological Studies Depression Scale (CES-D) [19]. This scale, commonly used for screening depression in previous research, was adapted to provide a

brief insight into depressive symptoms while minimizing the questionnaire burden. The selected items focused on symptoms of depression and anxiety, including fear, restlessness, and feelings of depression.

The scale responses were provided on a Likert scale with the following options: *rarely* (<1 day per week), *sometimes* (1–2 days), *very often* (3–4 days), and *mostly* (5–7 days).

A measure of respondents' resilience (3 items) was based on an adapted version of the Brief Resilience Scale, encompassing dimensions such as recovery, resistance, adaptation, and thriving [20]. The items included questions about difficulty in coping with stressful events caused by COVID-19, the ability to quickly adapt to COVID-19-related events, and challenges in returning to a usual lifestyle after controlling COVID-19. Responses were also based on a Likert scale with the following options: *strongly agree*, *agree*, *disagree*, and *strongly disagree*. A summated resilience score (range: 0–9) was calculated for each participant by assigning a weight of 0 to *strongly disagree*, 1 to *disagree*, 2 to *agree*, and 3 to *strongly agree*, and then summing the responses. Higher scores indicated greater resilience, with the scoring reversed for negatively worded items.

The survey was distributed, and the collected data remained anonymous. No geolocational data of the respondents were collected. The final survey was composed and administered in Arabic after a brainstorming session and a thorough review of relevant literature published in English. The survey development involved faculty from two pharmacy schools and one medical school. Initial brainstorming sessions were followed by focused discussions among researchers.

Since the items, concepts, and ideas originated in English, relevant scales identified in the literature were used to develop the survey in English before translation. The selected items were translated into Arabic using a back-translation methodology to ensure accuracy. One of two faculty members fluent in both Arabic and English, with experience in translating research materials, translated the English items into Arabic. This process avoided literal translations, ensuring that the meaning was clear and consistent with the original English items. The second faculty member, also fluent in both languages, performed an independent back-translation into English. This allowed the two English versions to be compared, ensuring the accuracy and retention of the original concepts and ideas.

The developed survey underwent review by additional faculty members within the relevant research area. Feedback from these reviews was incorporated, and minor revisions were made to finalize the survey.

The survey was pilot tested with 10 diabetic patients. During this phase, participants were asked to think aloud as they responded to the survey items. This allowed researchers to identify potential issues with the translated items and ensure their accuracy, clarity, and cultural appropriateness. Feedback from the pilot testing was used to make minor modifications to the survey.

Validity and Reliability Assessments

Several assessments were conducted to confirm the validity and reliability of the final survey used in the study. These included internal consistency analysis, the content validity index (CVI), and principal component analysis (PCA).

- **Internal Consistency**:

Internal consistency was assessed using Cronbach's alpha, which was 0.801. This value is acceptable as it exceeds the cut-off point of 0.6.

- **Face and Content Validity**:

Faculty members conducted face and content validity tests to ensure the quality of the survey instrument.

  - *Face validity*: Faculty members evaluated whether the survey items appeared to measure the intended concepts and were understandable and relevant to the research questions.
  - *Content validity*: Faculty members assessed whether the survey items adequately represented the constructs under study.

The content validity index (CVI) for the survey was

calculated based on feedback from six faculty members from the School of Pharmacy with clinical experience and postgraduate qualifications. The resulting CVI was 0.910, indicating excellent content validity.

- **Principal Component Analysis (PCA)**:

PCA was conducted to establish the validity of the instrument measuring the impact of COVID-19 on the mental health of diabetic patients. The sample size was deemed adequate based on the Kaiser-Meyer-Olkin (KMO) measure of 0.893, which exceeds the acceptable threshold of 0.6. Bartlett's test of sphericity yielded a p-value of <0.001, confirming the suitability of PCA.

In the PCA, three factors were retained based on the scree plot, representing the instrument's domains. The variance explained by the instrument was 56.3%, and high item loadings (>0.4) supported the domain labels. No items had low communalities (i.e., less than 0.3).

Sample Size

The target sample size of 368 responses was calculated to provide a representative sample of diabetic patients in Jordan. This calculation assumed a 5.11% margin of error and a 95% confidence interval, using an online sample size calculator (http://www.raosoft.com/).

Ethical Approval and Consent

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of King Abdullah University Hospital in Irbid, Jordan (IRB reference number: 2020/428). Patient consent to participate in the study was obtained through a consent form embedded within the survey. Respondents who did not agree to participate were unable to access the survey.

Data Analysis

Standard statistical methodologies were employed to assess the impact of COVID-19 on the mental health of diabetics. Statistical analyses were conducted using SPSS.

- **Descriptive Statistics**:

Descriptive statistics were used to summarize the data.

- **Multivariate Analysis**:

A multivariate model was used to identify factors associated with the impact of COVID-19 on the mental health of diabetic patients. Univariate analysis was conducted using the Chi-square test and independent-sample *t*-test, depending on variable distribution. Statistically significant variables were included in a backward logistic regression model to identify independent predictors.

- **Correlation and Group Comparisons**:

Pearson correlation coefficients were calculated to examine relationships among the resilience, fear, and anxiety/depression scales. One-way ANOVA was performed to compare the means of the fear and anxiety/depression scales across employment status groups. Statistical significance was set at $p = 0.05$.

RESULTS

A total of 371 surveys were completed. The majority (70.9%) of the respondents were between the ages of 41 and 64 years. There were more male respondents than female respondents. A total of 37.7% of the respondents were employed, and one-quarter were retired. The majority (83.4%) of the respondents lived in urban areas. Medical insurance covered 66.8% of the respondents, and 52.8% had only completed secondary education. Chronic disease was present in 69.3% of the respondents, and the majority (76.8%) reported good health status. Table 1 summarizes the demographic variables of the respondents.

The prevalence and causes of worry among diabetics in relation to the COVID-19 pandemic are summarized in Table 2. A total of 82.5% of respondents were very worried about the economic recession, and 78.2% were concerned about the health of their loved ones. Almost 74% of respondents were afraid of losing someone they care about due to COVID-19, 73% were concerned about not being able to pay for their basic needs, and 72.5% were worried about limited freedom (movement restrictions). Furthermore, 66.1% of respondents were concerned about increased pressure on the health system, and 63% were worried about difficulties accessing food supplies.

Additionally, 58.3% of respondents were concerned about losing their job or being laid off. Almost half of the participants were also worried about their physical and mental health, the spread of COVID-19, and the number of deaths reported in the media.

In addition to worry, Table 2 illustrates the anxiety and depressive symptoms related to the COVID-19 pandemic over the past two months. A total of 36.3% of respondents experienced depressive symptoms three days per week or more, and 26.1% felt depressed even when family and friends provided support three days per week or more. Regarding anxiety symptoms, 30.5% of respondents felt fear three days per week or more, 36.7% experienced restlessness three days per week or more, 35.3% felt tense or anxious about the pandemic three days per week or more, and 28.5% were unable to stop or control their worry three days per week or more.

Table 1: General characteristics of the study sample

Variable		Frequency (%)
Age (year)	Less than 40	76 (20.5)
	41-64	263 (70.9)
	above 65	263 (8.6)
employment status	Yes	140 (37.7)
	No	123 (33.2)
	Student	17 (4.6)
	Retired	91 (24.5)
Area of living	Rural	61 (16.4)
	Urban	310 **(83.6)**
Medical insurance	Yes	248 (66.8)
	No	123 (33.2)
Levels of Education	School (primary and secondary)	196 (52.8)
	College degree	64 (17.3)
	University degree	74 (19.9)
	High studies	37 (10)
Chronic diseases	Yes	257 **(69.3)**
	No	114 (30.7)
Chronic diseases	HTN, CVD	123 (48.1)
	Thyroid disorder	5 (1.9)
	Kidney disease	7 (2.6)
	Hyperlipidemia	104 (40.5)
	Asthma, COPD	17 (6.6)
Health status	Very bad	9 (2.4)
	Bad	50 (13.5)
	Good	285 **(76.8)**
	Very good	27 (7.3)

Table 2 also presents the degree of resilience of the responders in relation to the COVID-19 pandemic and its effects. A total of 60.1% of the respondents reported that they agreed or strongly agreed that they had difficulty getting through stressful events caused by COVID-19. Additionally, 72.8% of the respondents agreed or strongly agreed that they could quickly adapt to the events of COVID-19, and 49.6% of the respondents agreed or

strongly agreed that they had difficulty returning to their usual lifestyle after controlling COVID-19.

Correlation analysis revealed that those who had more resilience to stress experienced lower fear (Pearson coefficient = -0.238; p-value < 0.001). Those with more resilience to stress also had lower anxiety/depression (Pearson coefficient = -0.316; p-value < 0.001), and those who scored higher on the fear scale were more likely to have higher anxiety and depression (Pearson coefficient = 0.558; p-value < 0.001) (Table 3).

One-way ANOVA showed that the mean score for the fear scale was 7.03 for students, 9.07 for the unemployed, 9.72 for the employed, and 10.23 for retired participants (p-value < 0.001). The mean score for the anxiety/depression scale was 4.86 for students, 5.90 for the unemployed, 6.67 for the employed, and 9.41 for retired participants (Table 4).

Table 2: Emotions expressed by the diabetics related to COVID-19 pandemic

Prevalence and causes of worry feelings of the diabetics related to COVID-19 pandemic				
		Worry a lot N (%)	**Worry N (%)**	**Don't worry at all N (%)**
Losing someone you love as a result of corona		154 (41.5)	120 (32.3)	97 (26.1)
Increased pressure on the health system		77 (20.8)	168 (45.3)	126 (34.0)
Your physical and mental health		73 (19.7)	141 (38.0)	157 (42.3)
The health of your loved ones		135 (36.4)	155 (41.8)	81 (21.8)
Restricted freedom (restricted movement)		115 (31.0)	154 (41.5)	102 (27.5)
Economic recession		184 (49.6)	122 (32.9)	65 (17.5)
Difficulty obtaining food supplies		91 (24.5)	143 (38.5)	137 (36.9)
You become unemployed		139 (37.5)	77 (20.8)	155 (41.8)
Inability to pay for life		167 (45.0)	105 (28.3)	99 (26.7)
Scary messages in the media about the spread of COVID 19 and the number of deaths		53 (14.3)	147(39.6)	171 (46.1)
The degree of anxiety and depressive symptoms during the past two months				
	rarely (<1day) N (%)	**Sometimes (1-2 days) N (%)**	**very often (3-4 days) N (%)**	**mostly (7-5 days) N (%)**
Feeling depressed	123 (33.2)	113 (30.5)	84 (22.6)	51 (13.7)
Afraid	151 (40.7)	107 (28.8)	82 (22.1)	31 (8.4)
Feeling depressed even with family and friends help.	200 (53.9)	74 (19.9)	69 (18.6)	28 (7.5)
became restless	131 (35.3)	104 (28)	95 (25.6)	41 (11.1)
Feeling tense or anxious about the Corona epidemic	143 (38.5)	97 (26.1)	84 (22.6)	47 (12.7)
Not being able to stop or control worry	177 (47.7)	88 (23.7)	74 (19.9)	8.6)
The degree of resilience with the pandemic and its effects				
	Strongly agree N (%)	**Agree N (%)**	**Disagree N (%)**	**Strongly disagree N (%)**
Difficulty to get through the stressful events caused by COVID19	72 (19.4)	151 (40.7)	121 (32.6)	27 (7.3)
Ability to quickly adapt to the events of COVID19	78 (21)	192 (51.8)	73 (19.7)	28 (7.5)
Difficulty to go back to the usual lifestyle after controlling Corona.	75 (20.2)	109 (29.4)	123 (33.2)	64 (17.3)

Table 3 Results of correlation analysis using Pearson Correlation (Pearson coefficient)

scales	Fear scale (the higher values associated with higher fear) Mean score: 8.8 Maximal possible score: 20	Anxiety/depression scale (the higher values associated with higher anxiety and depression) Mean score: 6.1 Maximal possible score: 18
Resilience scale (higher values better resilience) Mean score: 4.6 Maximal possible score: 9	-0.238 (p-value <0.001)	-0.316 (p-value <0.001)
Fear scale	-	0.558 (p-value <0.001)

Table 4 results of the associations using one way ANOVA method

Employment status	Fear		Anxiety/Depression	
	Mean	p value	Mean	p value
Employed	9.72	<0.001	6.67	0.002
Unemployed	9.07		5.90	
Student	7.03		4.86	

Table 5 describes the independent predictors associated with depressive symptoms occurring more than 3 days per week during the past two months (COVID-19 pandemic period) using multivariate logistic regression analysis. The statistically significant independent predictors included younger age, difficulty accessing care during the COVID-19 pandemic, and anxiety symptoms lasting more than 3 days per week during the past two months.

Table 5: Independent predictors associated with depressive symptoms more than 3 days per week in the past two months, based on a single, self-rated item.

Variable	B (S.E.)	p value	Odds ratio	95% C.I. for odds ratio
Age	-0.024 (0.011)	0.03	0.977	0.956-0.998
Difficulty in access to care	0.703 (0.29)	0.015	2.02	1.145-3.565
Anxiety symptoms for more than 3 days per week	2.906 (0.3)	<0.001	18.284	10.164-32.889
Constant	-0.81 (0.596)	0.174	0.445	

DISCUSSION

Depressive and anxiety symptoms are among the most common emotional responses to stressful situations [21]. The current study aimed to assess the emotions provoked by the COVID-19 pandemic in diabetics using an online survey methodology. To address this health priority and meet the need for mental health data [22, 23], the study monitored mental health issues and found that one-quarter to one-third of the respondents experienced depressive and anxiety symptoms for 3 or more days per week during the

COVID-19 pandemic. The results of this study indicate a high burden on mental well-being due to the COVID-19 pandemic and associated interventions. It highlights the importance of implementing support systems and interventions to minimize the mental health impact of COVID-19 on patients with chronic diseases, as emphasized by health decision-makers and clinicians.

It is worth noting that resilience to stress is a protective factor present in some individuals, depending on their psychological makeup.

In situations like the COVID-19 pandemic, it is expected that feelings of fear can develop in individuals due to factors related to the seriousness, contagiousness, rapid spread, and high mortality of the virus [11]. The COVID-19 pandemic was classified as a Public Health Emergency of International Concern (PHEIC) [24, 25]. Surprisingly, the economic recession was found to be the most frequent source of worry among respondents. Typically, people fear dying, becoming sick (as reported in previous research), becoming helpless, or being stereotyped [26].

This finding can partly be explained by the fact that the majority of respondents were aged between 41 and 64. This demographic is more likely to be employed or own businesses, with their livelihoods heavily reliant on the economic system. Indeed, there have been significant negative effects on Jordan's economy arising from governmental interventions to address the COVID-19 pandemic. A review conducted by a research group found that, due to these governmental restrictions, many jobs were lost, leading to a reduction in the workforce.

Additionally, there was an increased demand for essential and COVID-19-related supplies, such as medical supplies, and a decreased demand for petroleum and manufactured products. Panic-buying has also been identified as a major contributing factor [27].

Due to the relatively low number of COVID-19 cases reported in Jordan, along with a lower number of deaths compared to other countries around the world [28] (Al-Smadi et al., 2021), the smallest proportion of worry in the present study was related to alarming media messages about the spread of COVID-19 and the number of deaths, despite the fact that most media messages were distressing. To maintain the viability of the economic system, the government did not enforce drastic infection control measures during the further waves of COVID-19 in Jordan. Thus, it is expected that feelings of worry would increase as a result of messages from the mass media. A follow-up investigation using a similar instrument could address this issue. It is well established that both traditional media and social media provide messages to the public that can inform health decisions, which are considered among the external factors affecting health beliefs. The quality of the former has been scrutinized in research studies due to the lack of control over the quality of reporting [29], while the latter is extensively criticized for major quality issues [30].

Depression and anxiety are common among individuals with chronic illnesses, such as diabetes, and it is expected that the COVID-19 pandemic would exacerbate this challenge. Anxiety and depressive emotions result from the stress posed by the rapid spread of the virus and fear of the future. These emotions are expected to vary depending on an individual's susceptibility to stress, pre-existing conditions (e.g., depression), resilience to stress, and genetic factors. Other overriding factors include lack of knowledge about infectious diseases and health behaviors, sleep problems, economic difficulties, inadaptability, problems with psychological coping strategies, and quarantine-related stress [31]. Depressive symptoms are further exacerbated in diabetics due to the increased risk of serious complications from COVID-19. Other notable mental health issues that can be exacerbated by the COVID-19 pandemic, as reported in research studies, include symptoms of psychosis, suicidal thoughts, post-traumatic stress, and panic attacks [32-36]. The prevalence of depressive and anxiety symptoms in the present study was

similar to that reported in a systematic review, where the pooled prevalence of anxiety was 31.9% and depression was 33.7% [31]. The prevalence was also similar to a study conducted in China [37]. Other studies reported an increase in negative emotions, such as anxiety and depression, due to the pandemic [38]. The prevalence of psychological consequences was also comparable to that observed in a previous study from Taiwan, which focused on the SARS outbreak [39].

Resilience to stress is a major mechanism for reducing the impact of stressful situations. Individuals with resilience are better able to cope with stress and are more immune to its effects [40]. These individuals typically put risks into perspective, using known facts to control their worries, meaning they do not worry illogically, nor do they neglect the worrying situation. In response to disease threats, people generally practice avoidant behaviors, such as social distancing and avoiding contact, with strict compliance to other interventions [41].

The results indicated that depressive symptoms were more common in younger individuals, which is surprising since elderly patients in the present study, who are all diabetic, have an increased risk for serious complications associated with COVID-19. This finding can partly be explained by the fact that increased depressive symptoms in younger individuals may be related to movement restrictions, preventing them from mingling and interacting with others. The streets being vacant and the restrictions on mood-lifting activities also likely contributed to this effect, as identified in a German study [25]. Studies from China and Germany have also highlighted that younger age is an independent predictor for generalized anxiety [25, 37].

Access to care plays an important role in maintaining health, and it can be affected by factors such as the high cost of care, particularly with decreased income due to quarantine measures and business closures, inconvenient healthcare facility locations, and decreased availability of telehealth services. Such barriers are expected to play a pivotal role in the development of depression and frustration, especially during times of crisis. Anxiety symptoms are often comorbid with depression, and it seems that increased anxiety can exacerbate depressive emotions, as identified in the present study.

The present study has a number of limitations. It did not address the severity of anxiety and depression symptoms, nor did it assess the impact of these ailments on social and occupational functioning. Such data would have enriched the study. Additionally, the methodology of data collection can impact the results. The data on anxiety and depression were collected using self-reported questions that provide only a snapshot of the emotions, rather than using a full-length questionnaire that assesses these emotions in more detail. The methodology was designed to meet the study objectives while minimizing the burden on respondents. Another limitation is the potential for selection bias, which can affect the generalizability of the results. The study used an online survey, which was inaccessible to individuals without internet access or those who are not familiar with using the internet. Lastly, the sample size calculation was based on a 5.11% margin of error, which might have had a small impact on the statistical power of the study.

The present study utilized a pre-tested online survey to assess key mental health issues during the COVID-19 pandemic within the diabetic patient population. This approach aimed to provide a comprehensive snapshot of the pandemic's influence on mental well-being. The findings are envisioned to offer insights into trends in mental health conditions during crises, which could inform future research in this area.

The study highlighted the primary concerns and mental health status of diabetic patients, revealing significant implications for clinical practice. The identified issues could be used to formulate targeted interventions for this patient group during crises, emphasizing the need for holistic healthcare services that address both physical and mental health.

Moreover, the study presents a valuable opportunity for healthcare policymakers, clinicians, and other stakeholders to gather baseline data for future disease outbreaks. This data could enable better preparation for identifying emerging mental health issues and support the development of policies and procedures that integrate mental health well-being into crisis response strategies.

CONCLUSION

The present study aimed to assess the impact of the COVID-19 pandemic on anxiety and depressive symptoms in patients with diabetes. The largest cause of worry for the respondents was the economic recession, followed by concerns about the health of their loved ones and the fear of losing someone they love. The smallest cause of worry was frightening messages in the media about the spread of COVID-19 and the number of deaths. A considerable proportion of respondents reported experiencing depressive and anxiety symptoms three days per week or more during the COVID-19 pandemic. A high proportion of patients found it difficult to cope with the stressful events caused by the COVID-19 pandemic.

Independent predictors associated with depressive symptoms (three days or more per week during the COVID-19 pandemic), identified using multivariate logistic regression analysis, were younger age, difficulty in accessing care, and anxiety symptoms. The present study investigated several mental health issues related to the COVID-19 epidemic. It highlights the potential need to integrate mental health support into the routine care of diabetic patients, particularly during crises, through well-designed, tailored interventions to promote mental health, wellness, and resilience. Public health messages should also be carefully formulated to mitigate the stress and fear caused by crises.

REFERENCES

1. King H., Aubert R.E. and Herman W.H. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. *Diabetes care*. 1998; 21(9):1414-1431.
2. King H. and Rewers M. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. *Diabetes care*. 1993; 16(1):157-177.
3. Anderson R.J., et al. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes care*. 2001; 24(6):1069-1078.
4. Zahid N., et al. Depression and diabetes in a rural community in Pakistan. *Diabetes research and clinical practice*. 2008; 79(1):124-127.
5. Brown G.C., et al. Quality of life associated with diabetes mellitus in an adult population. *Journal of Diabetes and its Complications*. 2000; 14(1):18-24.
6. Ciechanowski P.S., Katon W.J. and Russo J.E. Depression and diabetes: impact of depressive symptoms on adherence, function, and costs. *Archives of internal medicine*. 2000; 160(21):3278-3285.
7. Egede L.E., Diabetes, major depression, and functional disability among US adults. *Diabetes care*. 2004;27(2):421-428.
8. Rubin R.R. and Peyrot M. Quality of life and diabetes. *Diabetes/metabolism research and reviews*. 1999; 15(3):205-218.
9. Zhang W. and Sullivan P.W. Minor depression and health status among US adults with diabetes mellitus. *Am J Manag Care*. 2007; 13:65-72.
10. Bruce D., et al. A prospective study of depression and mortality in patients with type 2 diabetes: the Fremantle Diabetes Study. *Diabetologia*. 2005; 48(12):2532-2539.
11. Surveillances V. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19)—China, 2020. *China CDC weekly*. 2020; 2(8):113-122.
12. Person B., et al. Fear and stigma: the epidemic within the SARS outbreak. *Emerging infectious diseases*. 2004; 10(2):358.

13. Tao N., An analysis on reasons of SARS-induced psychological panic among students. *Journal of Anhui Institute of Education*. 2003; 21(2):78-9.
14. Association A.D. How COVID-19 Impacts People with Diabetes. 2020.
15. Al Jomaa E.E., Al Meslamani A. and Abazid H. A Comparative Cross-Sectional Study-Knowledge, behavior and psychological change among Medical and Non-medical Students in Jordan during COVID-19 pandemic. *Jordan Journal of Pharmaceutical Sciences*. 2022; 15(2):204-213.
16. Jarab A.S., et al. Impact of Distance Learning on Pharmacy and Pharm. D Undergraduates' during the COVID-19 Pandemic in Jordan. *Jordan Journal of Pharmaceutical Sciences*. 2022; 15(3):355-364.
17. Al-Rahamneh H., et al. Quality of life in disabled versus able-bodied individuals during COVID-19 pandemic. *Jordan Journal of Pharmaceutical Sciences*. 2022; 15(2):227-238.
18. McCarthy-Larzelere M., et al. Psychometric properties and factor structure of the Worry Domains Questionnaire. *Assessment.* 2001; 8(2):177-191.
19. Lewinsohn P.M., et al. Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. *Psychology and aging.* 1997; 12(2):277.
20. Smith B.W., et al. The brief resilience scale: assessing the ability to bounce back. *International journal of behavioral medicine*. 2008. 15:194-200.
21. Vameghi R., et al. Path analysis of the association between socio-economic status, anxiety, perceived stress, social support and women's depression. *International Journal of Women's Health and Reproduction Sciences*. 2018; 6(3):283-289.
22. Rajkumar R.P., COVID-19 and mental health: A review of the existing literature. *Asian journal of psychiatry*. 2020; 52:102066.
23. organization W.h. Substantial investment needed to avert mental health crisis. 2020.
24. Health W. and Organization. Statement on the second meeting of the International Health Regulations Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV); 2020.
25. Bäuerle A., et al. Increased generalized anxiety, depression and distress during the COVID-19 pandemic: a cross-sectional study in Germany. *Journal of Public Health*. 2020; 42(4):672-678.
26. Hall R.C., Hall R.C. and Chapman M.J. The 1995 Kikwit Ebola outbreak: lessons hospitals and physicians can apply to future viral epidemics. *General hospital psychiatry*. 2008; 30(5):446-452.
27. Nicola M., et al. The socio-economic implications of the coronavirus pandemic (COVID-19): A review. *International journal of surgery*. 2020; 78:185-193.
28. Al-Smadi A.M., et al. Public knowledge, attitudes, and practice about COVID-19 pandemic. *Journal of Public Health in Africa*. 2021; 12(2).
29. Coomber R., Morris C. and Dunn L. How the media do drugs: Quality control and the reporting of drug issues in the UK print media. *International Journal of Drug Policy*. 2000; 11(3):217-225.
30. Purcell G.P., Wilson P. and Delamothe T. The quality of health information on the internet: As for any other medium it varies widely; regulation is not the answer. *British Medical Journal Publishing Group*. 2002; 557-558.
31. Salari N., et al. Prevalence of stress, anxiety, depression among the general population during the COVID-19 pandemic: a systematic review and meta-analysis. *Globalization and health*. 2020; 16(1):1-11.
32. Organization W.H., Mental health and psychosocial considerations during the COVID-19 outbreak, 18 March 2020. World Health Organization. 2020.
33. Taylor M.R., et al. Factors influencing psychological distress during a disease epidemic: data from Australia's first outbreak of equine influenza. *BMC public health*. 2008; 8(1):1-13.

34. Ahmed M.Z., et al. Epidemic of COVID-19 in China and associated psychological problems. *Asian journal of psychiatry*. 2020; 51:102092.
35. Cao W., et al. The psychological impact of the COVID-19 epidemic on college students in China. *Psychiatry research*. 2020; 287:112934.
36. Wang Y., et al. Study on the public psychological states and its related factors during the outbreak of coronavirus disease 2019 (COVID-19) in some regions of China. *Psychology, health & medicine*. 2021; 26(1):13-22.
37. Huang Y. and Zhao N. Generalized anxiety disorder, depressive symptoms and sleep quality during COVID-19 outbreak in China: a web-based cross-sectional survey. *Psychiatry research.* 2020; 288:112954.
38. Li S., et al. The impact of COVID-19 epidemic declaration on psychological consequences: a study on active Weibo users. *International journal of environmental research and public health*. 2020; 17(6):2032.
39. Su T.-P., et al. Prevalence of psychiatric morbidity and psychological adaptation of the nurses in a structured SARS caring unit during outbreak: a prospective and periodic assessment study in Taiwan. *Journal of psychiatric research.* 2007; 41(1-2):119-130.
40. Jackson D., Firtko A. and Edenborough M. Personal resilience as a strategy for surviving and thriving in the face of workplace adversity: a literature review. *Journal of advanced nursing*. 2007; 60(1):1-9.
41. Schaller M., Murray D.R. and Bangerter A. Implications of the behavioural immune system for social behaviour and human health in the modern world. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2015; 370(1669):20140105.

تأثير جائحة كوفيد-19 على الصحة النفسية لمرضى السكري في الأردن: دراسة مسحية إلكترونية

***غيث الطعاني*[1]، صهيب مفلح[2]، روان الشريدة[3]، ريما كراسنة[4]، ساير العزام[2]**

[1] قسم الصيدلة السريرية والممارسة الصيدلانية، كلية الصيدلة، جامعة اليرموك، اربد – الأردن.

[2] قسم الصيدلة السريرية، كلية الصيدلة، جامعة العلوم والتكنولوجيا الأردنية، اربد – الأردن.

[3] قسم الصيدلانيات والتقنيات الصيدلانية، كلية الصيدلة، جامعة اليرموك، اربد – الأردن.

[4] قسم العلوم الطبية الأساسية، كلية الطب، جامعة اليرموك، اربد – الأردن.

ملخص

هدفت هذه الدراسة الى تقييم تأثير جائحة كوفيد-19 على الصحة العقلية لمرضى السكري. تم تطوير استبيان و تم اختباره مسبقًا لتقييم القلق وأعراض الاكتئاب ومرونة مرضى السكري و تم توزيعه عبر الانترنت. تم جمع البيانات من 371 مريض سكري. تتراوح أعمار غالبية المستجيبين (71.2%) بين 41 إلى 64 عامًا. وكانت الأمراض المزمنة موجودة لدى 69.3% من المشاركين، في حين أشار 76.8% إلى أنهم يتمتعون بصحة جيدة بشكل عام. وأعرب نحو 80% من المشاركين عن قلقهم بشأن الركود الاقتصادي، بينما أبدى 20% الباقين قلقهم بشأن الرسائل الإعلامية المخيفة. حوالي 35% ممن شملهم الاستطلاع عانوا من أعراض الاكتئاب، و30.5% كانوا يعانون من القلق. وافق ما يقرب من 60% من المشاركين أو وافقوا بشدة على أنهم واجهوا صعوبة في اجتياز الأحداث الضاغطة خلال جائحة كوفيد-19. وكشفت النتائج عن مدى قلق مرضى السكري بشأن حالة صحتهم العقلية، وهي معلومات قيمة لواضعي السياسات الصحية ومقدمي الرعاية الصحية.

الكلمات الدالة: كوفيد-19، الصحة العقلية، السكري، الاردن

* ***المؤلف المراسل: غيث الطعاني***

g.altaani@yu.edu.jo

تاريخ استلام البحث 2024/01/14 وتاريخ قبوله للنشر 2024/06/13.

Can Intravenous Lipid Emulsion Reduce Mortality Rate in Cases of Acute Aluminium Phosphide Poisoning? A Systematic Review

Howaida Saeed Mohammed[1*], Rahma Mahmoud Saad[2], Ahmed Tharwat Al-Namrouti[3], Reem Ihab Farouk[4], Samar Ramadan Mohamed AbdelGhani[1], Hanan Elimam[5]

[1]Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Cairo University, Kasr Alainy Street, Cairo, Egypt.
[2]Faculty of medicine, modern University for technology and information, Egypt.
[3]Al Galala faculty of medicine, Cairo University, Egypt.
[4]Clinical pharmacy, Faculty of Pharmacy, Cairo University, Egypt.
[5]Biochemistry, faculty of pharmacy, University of Sadat City, Egypt.

ABSTRACT

Background: Acute pesticide poisoning has remained a significant public health concern for decades. Supportive care has been the mainstay of treatment. Intravenous lipid emulsion (ILE) therapy offers a potential new strategy.
Objectives: This systematic review aimed to evaluate the current research on the efficacy of ILE in treating aluminum phosphide (AlP) poisoning.
Methods: A comprehensive electronic search was conducted across various databases including PubMed, Cochrane Central Register of Controlled Trials (CENTRAL), Campbell Systematic Reviews, Scopus, Web of Science, Springer Nature, Elsevier, Google Scholar, and regional databases encompassing Mansoura, Zagazig, Ain Shams universities, and Indian publications. Studies published in English language were considered for inclusion (from 2015 to 2023). Inclusion criteria focused on human studies evaluating the use of ILE for AlP intoxication.
Results: Five studies met the inclusion criteria, three studies were randomized controlled trials, one was observational cross sectional study**,** and one was case report encompassing a total of 224 patients. Of these, 102 patients received ILE, with all studies utilizing 20% ILE. Three studies administered ILE as a continuous intravenous infusion at a rate of 10 mL/h. Two other studies employed a bolus dose regimen, ranging from 1-3 mL/kg delivered over one minute, followed by continuous infusion. The overall mortality rate was 68.6% in the ILE group compared to 76.2% in the control group and the need for mechanical ventilation was lower in the ILE group with clinical improvement in the ILE group.
Conclusion: Intravenous lipid emulsion represents a novel therapeutic approach in toxicology with the potential to improve patient outcomes. This review suggests ILE may reduce mortality associated with AlP poisoning. Additionally, ILE use might be associated with decreased, need for mechanical ventilation, hospital stay and discharge time among survivors.
Key words: Aluminium; Phosphide; Intravenous; Emulsion; Treatment.

INTRODUCTION

For many years, acute pesticide poisoning has been regarded a significant public health issue [1]. According to the systematic review published at the year 2020 [2], 385 million unintentional acute pesticide poisonings were reported each year, resulting in 11,000 deaths globally.

Aluminium phosphide (AlP) known as wheat pill in the Egyptian market, is a well-known pesticide and rodenticide. It is used in several Asian nations, including

[*]***Corresponding author: Howaida Saeed Mohammed***
mhowaida51@kasralainy.edu.eg
Received: 04/02/2024 Accepted: 13/06/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2329

© 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

Pakistan and India, to protect wheat, rice, and other grains against rats. AlP is available in a variety of forms beyond tablets, including pellets, granules, and powders containing dark yellow crystals. Toxic phosphine (PH3) is released when AlP is exposed to moisture or water. Aluminium phosphide (AlP) acts as a pesticide to safeguard agricultural products. The matching response is as follows: AlP + 3H+ → Al3+ + PH3; AlP + 3H+ → Al (OH)3 + PH3 [3].

Exposure to phosphine triggers the production of reactive oxygen species (ROS), which are believed to be a major factor in phosphine poisoning. Studies have shown a link between phosphine treatment and lipid peroxidation, a process damaging cell fats, in all organisms tested, from plants to mammals. This damage, particularly from byproducts like 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA), can disrupt various enzyme functions essential for core metabolic processes. Notably, ROS can both cause and be caused by lipid peroxidation, suggesting these two processes work together to worsen phosphine toxicity [4].

Supportive measures well known to be the main treatment of Aluminium phosphide toxicity [5]. Moreover, magnesium sulfate, melatonin, N-acetylcysteine, glutathione, sodium selenite, vitamins C and E, triiodothyronine, liothyronine, vasopressin, milrinone, Laurus nobilis L., 6-aminonicotinamide, boric acid, acetyl-L-carnitine, and coconut oil, were suggested through reported experimental and clinical studies that they can be used as antidotes by reducing the noxious oxidative characters of ALP [6].

Intralipid emulsion (ILE) has been alternatively, utilized to treat different lipophilic poisoning despite its primary application for toxicities related to local anesthetics. Infusion of ILE has been proposed by many previous studies as a method that can generate a lipid sink that would allow a toxin with lipid soluble properties, like phosphine, to be trapped inside the lipid emulsion and reduce both its toxicity and effect site concentration [7, 8]. Moreover, recent research suggests that ILE enhances cardiac cell survival and has a direct inotropic effect [9-12]. Furthermore, ILE is hypothesized to enhance mitochondria's uptake of fatty acids, permitting the restoration of ATP stores in spite of the harmful suppression of fatty acid transport systems [13-16].

In Egypt, practically little has been done to conduct scientific research on this subject and stop the continued loss of innocent lives, despite the rising number of patients who are hospitalized every year due to poisoning. Nevertheless, Egypt has paid close attention to the high number of poisoning-related deaths and to the poor survival rate after ALP poisoning.

This study aimed to provide a systematic review of the literature on the role of intravenous lipid emulsion in treating Aluminium phosphide toxicity. By identifying the drug's effective dose and ability to lower the mortality rate, this information may help develop clear guidelines for the treatment of such deadly poisoning.

METHODS

Search strategy:

An electronic search was carried out in the following databases, Pubmed, Cochrane Register of Controlled Trials (CENTRAL), Campbell Systematic Reviews, Scopus, Web of Science, Springer Nature, Elsevier, Google Scholar, Ovid, Mansoura journals, Zagazig journals, Ain Shams journals, and Indian journals. The search strategy employed a combination of Medical Subject Headings (MeSH) terms and keywords tailored to the study objectives and inclusion criteria. The following terms were used: IV lipid emulsion AND Aluminium AND phosphide AND (toxicity OR poisoning) AND (treatment OR management) were used for search in this database. The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines were used [17].

Study selection and data extraction:

Studies were selected based on pre-defined inclusion criteria. Two reviewers independently screened titles and

abstracts of identified studies. Full-text articles were retrieved for studies deemed potentially relevant based on initial screening. Discrepancies between reviewers were resolved through discussion or by consulting a third reviewer

Inclusion Criteria:

- Studies investigating the use of intravenous lipid emulsion (ILE) in conjunction with standard care for the treatment of aluminum phosphide (AlP) poisoning.
- Studies with a control group receiving standard treatment in the intensive care unit (ICU) setting.
- Studies enrolling patients with AlP intoxication who remained hemodynamically unstable despite receiving all supportive care measures.
- Studies reporting mortality rates as an outcome measure.

Exclusion Criteria:

- Studies solely investigating the use of ILE for other indications or AlP toxicity without incorporating ILE therapy.
- Studies published in languages other than English.
- Reviews, letters to the editor, or editorials.

Ethical Approval

Ethical approval for this study was obtained from the scientific committee of the Forensic Medicine and Clinical Toxicology department and the ethical committee of Kasr Alainy Faculty of Medicine, Cairo University (Approval number: 507-2023).

Data extraction:

To minimize bias, four researchers extracted data from the included studies, working independently of each other. Two additional researchers independently reviewed the extracted data for discrepancies, which were resolved through discussion and consensus. The following information was collected from each study:

Outcomes:

- **Primary:**
  - Changes in mortality and morbidity rates, including cardiotoxicity, hepatotoxicity, metabolic disorder, and others.
- **Secondary:**
  - Length of hospitalization for survivors and non-survivors.
  - Need for mechanical ventilation.

Evaluation of study methodological quality

To assess the risk of bias within the included studies, a methodological quality evaluation was performed. Randomized controlled trials (RCTs) were evaluated using the Cochrane risk-of-bias tool for randomized trials (Rob 2) as outlined in the Cochrane Handbook of Systematic Reviews of Interventions [18]. This tool assesses bias across five key domains:

- Selection bias: Evaluates the potential for bias introduced during the randomization process.
- Performance bias: Assesses the potential for bias introduced by deviations from the intended interventions for either the ILE or the control group.
- Detection bias: Evaluates the potential for bias introduced by incomplete or inaccurate measurement of outcomes.
- Attrition bias: Assesses the potential for bias introduced by missing outcome data due to participant dropout.
- Reporting bias: Evaluates the potential for bias introduced by selective reporting of outcomes.

For non-randomized observational studies, the Newcastle-Ottawa quality assessment scale (NOS) [19] was used to assess methodological quality. Cross-sectional and case report studies were evaluated using Joanna Briggs Institute [JBI] Critical Appraisal Checklist for case reports [20] for case reports.

Risk of bias of included studies:

The risk of bias within the included studies was evaluated using appropriate tools based on study design:

- Randomized Controlled Trials (RCTs): The Cochrane risk-of-bias tool for randomized trials (Rob 2) was employed to assess bias across key domains

according to the recommendations in the Cochrane Handbook of Systematic Reviews of Interventions [18].

- **Observational Studies:** The Newcastle-Ottawa quality assessment scale (NOS) [19] was utilized to evaluate the methodological quality of observational studies.
- **Cross-Sectional and Case Report Studies:** A relevant quality assessment tool, Joanna Briggs Institute [JBI] Critical Appraisal Checklist for case reports [20] was employed to assess the methodological quality of these studies.

The assessment revealed the following distribution of risk of bias within the included studies:

- One randomized controlled trial (RCT) exhibited a high risk of bias [21].
- One study demonstrated a medium risk of bias [22].
- The remaining studies displayed a low risk of bias [23, 24, 25].

Results

Search Methods:

A systematic search identified 461 relevant articles from PubMed, Cochrane, Scopus, Web of Science, Google Scholar, and Ovid databases. After removing duplicates and screening titles and abstracts, five studies met the inclusion criteria (Figure 1).

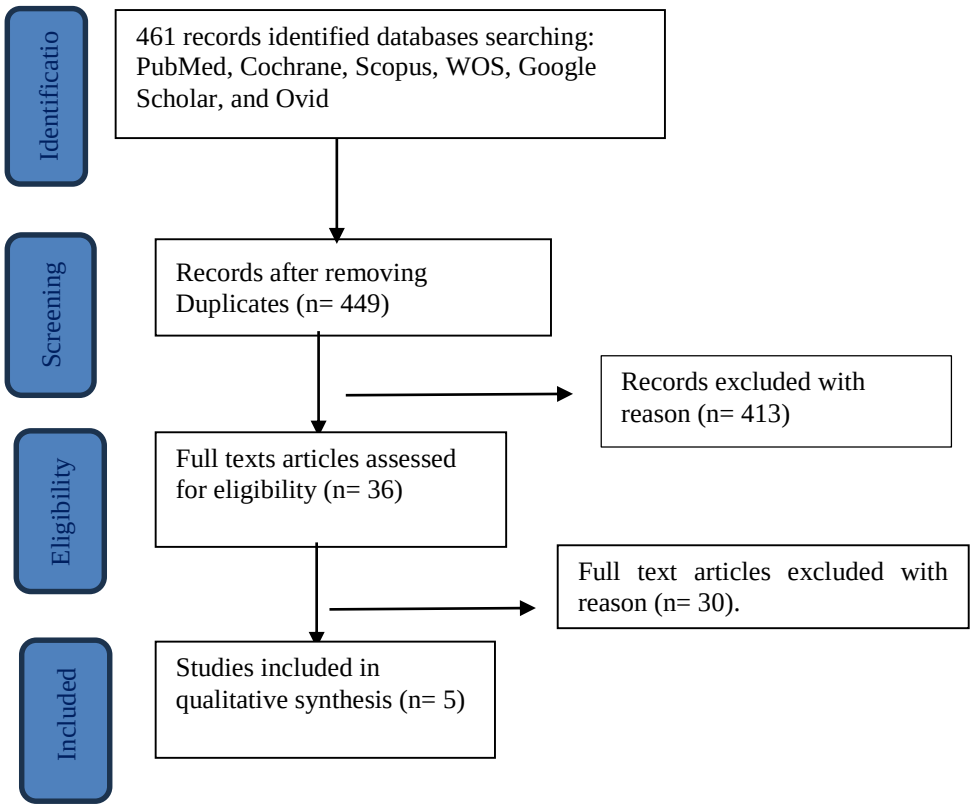


Figure 1: PRISMA flowchart of systematic review.

Study Design:

Three studies were randomized controlled trials (RCTs) [21, 23, 25], one was an observational cross-sectional study [24], and the last was a case report [22] (Table 1). The total sample size was 224 patients. The ILE group included 102 patients (59 females, 40 males), with unclear sex data for three patients in the control group [24]. Three RCTs were conducted in Egypt [21, 23, 25], with one each from Iran and India [22, 24]. The age of the majority of cases ranged from 20 to 40 years [22-25], except one study [21] the majority was <20 years (Table 2).

Table (1): General characteristics (type, duration and country) of the studies.

Authors	Country	Type of the study	Duration
Torabi et al (2023)	Iran	Cross sectional	……
Taalab et al (2022)	Egypt	Randomized Controlled Trial	January 2016 to January 2018
Hussein et al (2021)	Egypt	Randomized Controlled Trial	From the beginning of October 2020 to the end of January 2021
ELabdeen et al (2020)	Egypt	Randomized Controlled Trial	December 2016 till November 2017
Baruah (2015)	India	Case report	……

Table (2): Sample size, age and sex of patients included in each study.

Authors	Sample size (total N = 224)			Age		Sex	
Torabi et al (2023)	Total 19	Case 3	Control 16	M=31.79±10.97 years (15 – 66y)		Females = 8 (42.1%) Males = 11 (57.9%)	
Taalab et al (2022)	87	41	46	M = 28.23 ± 9.69 (13- 47y)		Females = 56 (64.4%) Males = 31 (35.6%)	
				Case ≤20 years=7 21-40 years=26 41-60y =8	Control ≤20 y=16 21-40 y=27 41-60y=3		
Hussein et al (2021)	66	31	35	……..		Case Female = 58.1% Male = 41.9%	Control 60% 40%
ELabdeen et al (2020)	50	25	25	Case <20y=14 20-30y=3 >30-50=8	Control <20y=10 20-30y=7 >30-50=8	Case Females = 17 Males = 8	Control 14 11
Baruah (2015)	2	2	------	Female =40 y Male =30 y		1 female 1 male	
Total	224	102	122				

M = Mean.

N= Number.

Intravenous Lipid Emulsion Dosing:

All five studies used 20% ILE. Three used a dosage of 10 mL/hour intravenous (IV) infusion [21, 22, 24]. One study administered an initial bolus dose of 1-3 mL/kg starting 5-20 minutes after poisoning, repeated 2-3 times every 5 minutes up to 300 ml for persistent cardiovascular collapse followed by a continuous infusion (0.25 ml/kg/min) with the option to double the dose (0.5 ml/kg/min) for persistent hemodynamic instability [23]. Another study used a 1.5 ml/kg bolus over one minute

followed by a continuous infusion (0.25 ml/kg/min for 30-60 minutes) and doubled to 0.5ml/kg/min permissible for continued hemodynamic instability when Blood pressure remain low, moreover the infusion was continued for at least 10 minutes after circulatory stability [25]. In the studies, the infusion was tapered gradually with monitoring of serum triglyceride level (Table 3).

Table 3 Results of correlation analysis using Pearson Correlation (Pearson coefficient)

scales	Fear scale (the higher values associated with higher fear) Mean score: 8.8 Maximal possible score: 20	Anxiety/depression scale (the higher values associated with higher anxiety and depression) Mean score: 6.1 Maximal possible score: 18
Resilience scale (higher values better resilience) Mean score: 4.6 Maximal possible score: 9	-0.238 (p-value <0.001)	-0.316 (p-value <0.001)
Fear scale	-	0.558 (p-value <0.001)

Primary Outcomes:

- Mortality Rate: The overall mortality rate was 68.6% (70/102 patients) in the ILE group compared to 76.2% (93/122 patients) in the control group, with a statistically significant difference between the groups. Cardiogenic shock was the main cause of mortality in AlP [25].
- Morbidity Rate: All studies reported morbidity as cardiotoxicity, hepatotoxicity, saturation and metabolic changes. Survivors had higher systolic pressure, higher diastolic pressure, higher heart rate, and higher percentage of oxygen saturation, in addition higher average PH, HCO3, and pCO2 with clinical improvement after administration of ILE infusion [21 – 25]. Furthermore, the initial cardiac arrest rhythm was shockable in the majority of survivors [23]. While, the heart rate was significantly lower, decreases in liver enzymes (SGOT and SGPT) and bilirubin, later on the haemodynamic compromise and metabolic acidosis caused by acute AlP poisoning improved [25] (Table 3).

Secondary Outcomes:

Discharge and Survival Time:

- **ElLabdeen et al., [21]** reported an average hospital stay of 60 hours for survivors in the ILE group compared to 98 hours in the control group.
- **Talaab et al., [23]** reported varying lengths of stay for survivors (6-24 hours, 5-48 hours, and 49-72 hours).
- **Torabi et al., [24]** reported a 10.5% (2 patients) discharge rate, with survivors having an ejection fraction of 30-50%, compared to deceased patients with an ejection fraction below 30%.
- The case report **[22]** documented a female patient discharged on day 5 and a male discharged on day 10.

Need for Mechanical Ventilation:

- Only ElLabdeen et al. [21] reported this outcome. The need for intubation and mechanical ventilation was significantly lower in the ILE group (36%) compared to the control group (92%). No other studies mentioned this outcome (Table 3).

DISCUSSION

Food production and agriculture also have a significant impact on health. Since the production of staple foods like grains and vegetables is needed for growth, energy, and maintaining good health, a strong agricultural system is vital to people's well-being and health [26].

Suicidal poisoning from pesticide toxicity is a highly prevalent and difficult situation, particularly in developing countries where sales of the poison are unrestricted. In recent years, the most popular means of suicide in Egypt, particularly in rural regions, has been the ingestion of wheat/rice tablets or aluminum phosphide (AlP) [27]. Up until now, supportive interventions have been the cornerstone of treatment for ALP toxicity and have been linked to high mortality rates. Therefore, it's critical to find alternate therapeutic approaches [28]. Hindering absorption or aborting systemic effect either by creating a lipid sink in blood or using antioxidant agents are under research. The purpose of this systematic review was to assess the safety and effectiveness of intravenous lipid emulsion in reducing mortality associated acute ALP poisoning.

The studies included in this review demonstrated gender disparity ((59 females and 40 males) among cases of acute Alp poisoning received ILE). It may be attributed to the fact that women are more likely than men to self-poison at a young age, either out of genuine suicidal intent or simply to win sympathy, as noted by Khan et al. [29]. Conversely, Mathai and Bhanu's (2010) reported a higher incidence of AlP poisoning in males, potentially due to easier access to fumigants through employment and the increased stress associated with financial burdens [30]. These findings suggest potential socio-demographic factors influencing the risk of AlP intoxication, warranting further investigation.

Apart from cases of local anesthetic toxicity, the ideal ILE dosage for acute intoxication is actually unclear. However, to best of our knowledge, the dosages utilized in the articles that are part of our analysis were regarded as safe and effective (not efficient). They were supposed to prevent potential ILE adverse effects, which are expected to manifest after a dose of 60 ml/kg and mostly impact the liver and lung tissues, which could offset the survival benefit [31].

The dosages utilized were consistent with the earlier findings by Macala and Tabrizchi (2014), which showed that doses over 1 mL/kg did not provide any extra advantages for treating lipophilic toxicity. However, ILE do not directly affect the heart at low dosages; instead, they primarily affect the pharmacokinetic profile of lipid-soluble toxins [32].

The initial intravenous bolus of 1.5 ml/kg over 1 minute is recommended by the 2015 European Resuscitation Council (ERC) Guidelines on Resuscitation, which are followed by a continuous infusion of 15 ml/kg/h. If there is ongoing circulatory collapse, the bolus should be given twice more at 5-minute intervals. The infusion ought to be maintained until the maximum dose of 12 ml/kg is reached or until hemodynamic recovery has been achieved. [31].

.Even though the observed increase in ALP poisoning survival did not reach a significant level in our systematic analysis, this might still be viewed as a positive finding because supportive care alone was insufficient to increase survival. This is corroborated by the findings of (Macala et al. (2018) that using ILE did not increase overall survival even when cardiac sodium channel blocking effect (as shown by QRS prolongation) improved [33]. In a similar way, ILE has demonstrated significant effectiveness in the resuscitation therapy of severe refractory systemic toxicity from local anesthetic drugs [34, 35]. Moreover, a recent literature review exploring novel therapies for AlP intoxication suggested a potential role for ILE in reducing mortality when combined with supportive care [3].

Comparing the effect of recently suggested antidotes on AlP poisoned patients survival, Goharbari et al. (2018) found that, as compared to control, patients who received 50 mg of liothyronine via nasogastric tube had a non-significantly lower mortality [36]. N-acetylcysteine has

been recognized as a potentially beneficial modality for AlP poisoning, as evidenced by recent systematic review and meta-analysis [37, 38]. The mortality rate of AlP patients who received N-acetylcysteine was non-significantly lower than that of the control group [39, 40]. However, a significant decrease in the mortality of the rabbits treated with a combination of trimetazidine, NAC and vitamin C was observed by El Shehaby et al. (2022) [41]. According to a clinical study, taking Coenzyme Q10 orally along with liquid paraffin oil can increase the survival rate of AlP intoxicated patients [42]. However, the pure consumption of Q10 and its effect in both invivo and invitro or clinical studies has never been investigated.

This indicate that, based on the information that is now available, it is not currently advised to utilize intralipids as a sole antidote for AlP intoxication. Therefore, if there is no suspicion of a negative drug interaction, it is preferable to employ a multiple treatment regimen beside supportive measures.

In the current review, according to the pooled effect assessment of the fixed-effect model, the intravenous lipid group differed significantly from the control group in terms of the length of hospital stay among survivors (shorter).This may reflect clinical improvement of AlP poisoned patients upon use of ILE.

Conversely, a study conducted by Darwish and his colleagues (2020) found that the duration of hospital stay was significantly longer in patients received Coenzyme Q10 orally along with liquid paraffin oil (G III) followed by patients received liquid paraffin (G II) and the shortest duration was in patients received gastric lavage with Kmno4 (G I). That could be explained by the higher percentage of survivors in groups II and III while most of the cases in group I died a short time after admission [42].

The current systematic review revealed that one study by El Labdeen et al. (2020) found that the experimental group required intubation and mechanical ventilation far less frequently than the control group (36% versus 92%, respectively) [21]. Given that endotracheal intubation was recommended in cases of AlP poisoning because of severe acidosis, significant myocardial suppression, pulmonary edema, aspiration, or disrupted sensorium, this could be a reflection of clinical improvement. Without a doubt, ILE's beneficial impacts in this regard reduce expenses, effort, and ventilation-related complications.

This is in line with Weinberg. (2012) findings that intubation for patients receiving lipid emulsion with multi- drugs overdose, including beta blockers, calcium channel blockers, tricyclic antidepressants, benzodiazepines, and other antidepressants, were shorter than those of matched controls [14].

Both traditional and modern medicine have been derived from plants all around the world. They have significantly improved human health and well-being by giving most people on the planet access to life-saving medications. Medicinal plants are used in traditional medicine, which treats more than 80% of the world's population, to treat a variety of illnesses, particularly in underdeveloped nations [43].

In the same line, Darwish et al 2020, reported high percentage (80%) of intubated patients in group I (only gastric lavage with Kmno4). This could point to the deterioration of the general condition of the patients in this group when compared to the other two groups. This denotes the relatively better outcome in these groups [42].

In the meantime, some research revealed that the production of reactive oxygen species (ROS) led to inflammation, which has been linked to the pathophysiological elements of a number of patient diseases. Therefore, this issue could be avoided by using the free radical scavenging method to scavenge ROS [44].

To our knowledge, no prior systematic review has been published on this subject. In order to gather data, we looked through four mega database websites. Upon reviewing the data from studies that contained our search terms, our systematic review estimated the mortality rate and morbidity, including the need for mechanical ventilation and hospital stays. Both are the two main

consequences of ALP poisoning.

Limitations and Weakness

This review is subject to several limitations as the paucity of research on this topic and the relatively small sample size of patients in the included studies. Therefore, the data available remains insufficient to draw firm conclusions about the effectiveness of ILE and generalizability of the findings in cases of AlP poisoning. Furthermore, the majority of studies originated from developing countries, which may be attributable to the prevalence of agriculture and the ready availability of the pesticides in these regions. Also, the majority of the studies completed up to this point that made up our systematic review omitted information about the laboratory results. The timing of administration affects the outcome as well, something that was not addressed in any of the systematic review papers. Moreover, Data from in-vitro studies were excluded, in the future their analysis could reveal the pathogenic impact of ILE on isolated organs.

CONCLUSION

This systematic review investigated the current body of research on the use of intravenous lipid emulsion (ILE) therapy in treating acute aluminum phosphide (AlP) poisoning. The review identified a limited number of studies, but the available evidence suggests a potential benefit for ILE in reducing mortality rates following AlP intoxication. While the findings are promising, further well-designed studies are required to definitively establish the efficacy of ILE therapy in this patient population.

RECOMMENDATIONS

- **Future research:**
  - Conduct large-scale, well-designed randomized controlled trials (RCTs) to definitively, assess the effectiveness of ILE therapy in reducing mortality associated with AlP poisoning.
  - Standardize ILE dosing regimens within RCTs to facilitate robust comparisons and evaluation of treatment efficacy.
  - Investigate the potential mechanisms by which ILE therapy may exert its beneficial effects in AlP poisoning.
- **Clinical practice:**
  - Consider the findings from this review when developing treatment protocols for AlP intoxication, particularly in settings with limited access to established therapies. However, due to the limitations of the current evidence base, ILE should not be used as a sole therapy but rather as a potential adjunct to supportive care measures.

Future Directions

The field of AlP poisoning management requires further exploration of novel therapeutic strategies. Future research should focus on developing and evaluating additional treatment modalities, alongside optimizing existing protocols, to improve patient outcomes in this life-threatening intoxication.

This systematic review identified a limited, but promising, body of research suggesting a potential role for ILE therapy in reducing mortality following AlP poisoning. Further studies are necessary to confirm these findings and establish optimal treatment protocols.

Funding: None.

Conflict of interest: Authors declare that there is no conflict of interest

REFERENCES

1. Eizadi-Mood N., Mahvari R., Akafzadeh S.M., Mohammadbeigi E., Feizi A., Mirmoghtadaei P., Sami R. and Meamar R. Acute pesticide poisoning in the central part of Iran: A 4-year cross-sectional study. *SAGE Open Med*. 2023; 7(11):20503121221147352. https://doi.org/10.1177/20503121221147352.
2. Boedeker W., Watts M., Clausing P. and Marquez E. The global distribution of acute unintentional pesticide poisoning: estimations based on a systematic review. *BMC Public Health*. 2020; 20(1): 1875. https://doi.org/10.1186/s12889-020-09939-0.
3. Naddafi M., Mehrizi A.A., Eghbal M.A., Khansari M.G. and Azarmi Y. Mohammad Reza Sattari a. Reducing the risk of death induced by aluminum phosphide poisoning: The new therapies. *Chemosphere*. 2022; 294:133800. https://doi.org/10.1016/j.chemosphere.2022.133800.
4. Shen X., Che M., Xu H., Zhuang X., Chen E.,Tang P. and Wang K. Insight into the molecular mechanism of phosphine toxicity provided by functional analysis of cytochrome b5 fatty acid desaturase and dihydrolipoamide dehydrogenase in the red flour beetle. *Tribolium castaneum Pesticide Biochemistry and Physiology*. 2023; 194:105482. https://doi.org/10.1016/j.pestbp.2023.105482.
5. Hassanian-Moghaddam H., Zamani N., Rahimi M., Hajesmaeili M.M. and Sadeghi R. Successful treatment of aluminium phosphidepoisoning by extracorporeal membrane oxygenation', *Basic and Clinical Pharmacology and Toxicology*. 2016; 118:243–246. https://doi.org/10.1111/bcpt.12481.
6. Karimani A., Mohammadpour A.H., Zirak M.R., Rezaee R., Megarbane B., Tsatsakis A., et al. Antidotes for aluminum phosphide poisoning – An update. *Toxicology Reports*. 2018; 5:1053–1059. https://doi.org/10.1016/j.toxrep.2018.10.009.
7. Rothschild Rothschild L., Bern S., Oswald S. and Weinberg G. Intravenous lipid emulsion in clinical toxicology. *Scand J Trauma Resusc Emerg Med*. 2010; 18:51-8. https://doi.org/ 10.1186/1757-7241-18-51
8. Muller S.H., Diaz J.H. and Kaye A.D. 'Clinical applications of intravenous lipid emulsion therapy. *Journal of Anesthesia*. 2015; 29: 920–926. https://doi.org/ 10.1007/s00540-015-2036-6
9. Stehr S.N., Ziegeler J.C., Pexa A., Oertel R., Deussen A., Koch T., et al. The effects of lipid infusion on myocardial function and bioenergetics in l-bupivacaine toxicity in the isolated rat heart. *Anesth Analg*. 2007; 104(1):186-92. https://doi:10.1213/01.ane.0000248220.01320.58. PMID: 17179268.
10. Partownavid P., Umar S., Li J., Rahman S. and Eghbali M. Fatty-acid oxidation and calcium homeostasis are involved in the rescue of bupivacaine-induced cardiotoxicity by lipid emulsion in rats. *Crit Care Med*. 2012; 40(8):2431-7. https://doi: 10.1097/CCM.0b013e3182544f48. PMID: 22647409; PMCID: PMC3401289.
11. Fettiplace M.R., Ripper R., Lis K., Lin B., Lang J., Zider B., et al. Rapid cardiotonic effects of lipid emulsion infusion*. *Crit Care Med*. 2013; 41(8):e156-e62. https://doi: 10.1097/CCM.0b013e318287f874. PMID: 23531591.
12. Fettiplace M.R., Lis K., Ripper R., Kowal K., Pichurko A., Vitello D., et al. Multi-modal contributions to detoxification of acute pharmacotoxicity by a triglyceride micro-emulsion. *J Control Release*. 2015; 198:62-70. https://doi: 10.1016/j.jconrel.2014.11.018. PMID: 25483426; PMCID: PMC4293282.

13. French D., Armenian P., Ruan W., Wong A., Drasner K., Olson K.R., et al. Serum verapamil concentrations before and after Intralipid therapy during treatment of an overdose. *Clin Toxicol (Phila)*. 2011; 49(4):340-4. https://doi: 10.3109/15563650.2011.572556. PMID: 21563913.
14. Weinberg G.L. 'Lipid emulsion infusion: resuscitation for local anesthetic and other drug overdose. *Anesthesiology*. 2012; 117(1):180-7. https://doi: 10.1097/ALN.0b013e31825ad8de. PMID: 22627464; PMCID: PMC4609208.
15. Ozcan M.S. and Weinberg G. Intravenous lipid emulsion for the treatment of drug toxicity. *J Intensive Care Med*. 2014; 29(2):59-70. https://doi: 10.1177/0885066612445978. Epub 2012 Jun 24. PMID: 22733724.
16. Murali R., Bhalla A., Singh D. and Singh S. Acute pesticide poisoning: 15 years experience of a large North-West Indian hospital. *Clin Toxicol (Phila).* 2009; 47(1):35-8. https://doi: 10.1080/15563650701885807. PMID: 18608278.R.
17. Moher D., Liberati A., Tetzlaff1 J. and Altman D.J. The PRISMA Group. METHODS OF SYSTEMATIC REVIEWS AND META-ANALYSIS. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Journal of Clinical Epidemiology*. 2009; 62:1006-1012. https://doi.org/10.1016/j.jclinepi.2009.06.005.
18. Higgins J.P.T., Thomas J., Chandler J., Cumpston M., Li T., Page M.J. and Welch V.A. (editors). *Cochrane Handbook for Systematic Reviews of Interventions version 6.4* (updated August 2023). Cochrane, 2023. Available from www.training.cochrane.org/handbook.
19. Wells G.A., Shea B., O'Connell D., Peterson J., Welch V., Losos M., et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses Department of Epidemiology and Commuunity Medicine, University of Ottawa, Room 3227A, 451 Smyth Road, Ottawa, Ontario K1J 8M5, Canada. https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (visited at 15/4/2024).
20. Moola S., Munn Z., Tufanaru C., Aromataris E., Sears K., Sfetcu R., et al. Chapter 7: Systematic reviews of etiology and risk. In: Aromataris E, Munn Z (Editors). *JBI Manual for Evidence Synthesis. JBI*, 2020. Available from https://synthesismanual.jbi.global (visited at 15/4/2024).
21. ELabdeen S.M.Z., Saad K.M., Oreby M.M. and Elgazzar F.M. Assessment of Intravenous Lipid Emulsion as an Adjuvant Therapy in Acute Aluminum Phosphide Poisoning: A randomized Controlled Trial. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology*. Jan 2020; 34:51-68. https://doi.org/10.1093/qjmed/hcaa049.001.
22. Baruah U., Sahni A. and Sachdeva H.C. Successful management of aluminium phosphide poisoning using intravenous lipid emulsion: Report of two cases. *Indian J Crit Care Med*. 2015; 19(12): 735-8. https://doi.org/10.4103/0972-5229.171412.
23. Taalab Y.M., Helmy M.A. and Othman A.A.A. Evaluation of the Role of Intravenous Lipid Emulsion as a Putative Treatment for Acute Aluminum Phosphide Poisoning. *Mansoura J. Forens. Med. Clin. Toxicol*. 2022; 30(1):71-84. https://doi.org/10.21608/mjfmct.2022.118125.1042.
24. Torabi M., Moridi Z.S. and Mirzaee M. The effect of intravenous lipid emulsion on the outcome of aluminium phosphide poisoning. *Med J Tabriz Uni Med Sciences*. 2023; 45(1):46 – 54. https://doi.org/10.34172/mj.2023.012.

25. Hussein H.A.M and Zidan D.H. Efficacy of intravenous lipid emulsion (ILE) in the treatment of acute aluminum phosphide toxicity at Alexandria Main University Hospital, Egypt. *Ann Clin Anal Med*. 2022; 13(1):93-97. https://doi.org/10.4328/ACAM.20863.
26. Dawood O.T. and Aladul M.I. Social Determinants of Health in Pharmacy Practice. *Jordan j. pharm. sci*. 2024; 17(3):629-637.:https://jjournals.ju.edu.jo/index.php/jjps/article/view/2364.
27. Hussein H.A.M. and Elsawaf H.E.M. Suicidal poisoning in Alexandria, Egypt-An updated statement. *Ann Clin Anal Med*. 2021; 12(8):888-894. https://doi.org/10.4328/ACAM.20484
28. Goharbari M.H., Taghaddosinejad F., Arefi M., Sharifzadeh M., Mojtahedzadeh M., Nikfar S., et al. Therapeutic effects of oral liothyronine on aluminum phosphide poisoning as an adjuvant therapy: A clinical trial. *Hum Exp Toxicol*. 2018; 37(2):107-117. https://doi.org/10.1177/0960327117694074. PMID: 29233028.
29. Khan A. and Ungar M. Resilience to self-harm: A scoping review of protective factors that aid in recovery among marginalized young people. *Crisis: The Journal of Crisis Intervention and Suicide Prevention*. 2021; 44(1): 61-69. https://doi.org/10.1027/0227-5910/a000831.
30. Mathai A. and Bhanu M.S. Acute aluminium phosphide poisoning: can we predict mortality? *Indian J Anaesth*. 2010; 54(4):302–307. https://doi.org/10.4103/0019-5049.68372.
31. Tampakis K., Vogiatzakis N., Kontogiannis C., Spartalis M., Ntalianis A., Spartalis E., et al. Intravenous lipid emulsion as an antidote in clinical toxicology: a systematic review. *Eur Rev Med Pharmacol Sci*. 2020; 24(12):7138-7148. https://doi: 10.26355/eurrev_202006_21708. PMID: 32633409.
32. Macala K. and Tabrizchi R. The effect of fat emulsion on hemodynamics following treatment with propranolol and clonidine in anesthetized rats. *Acad Emerg Med*. 2014; 21(11):1220-5. https://doi: 10.1111/acem.12511. PMID: 25377398.
33. Macala K.F., Khadaroo R.G., Panahi S., Gragasin F.S. and Bourque S.L. Low dose Intralipid resuscitation improves survival compared to ClinOleic in propranolol overdose in rats. *PLoS One*. 2018; 13(8):e0202871. https://doi: 10.1371/journal.pone.0202871. PMID: 30161186; PMCID: PMC6116996.
34. Weinberg G.L. Treatment of local anesthetic systemic toxicity (LAST). *Reg Anesth Pain Med*. 2010; 35(2):188-193. https://doi: 10.1097/AAP.0b013e3181d246c3. PMID: 20216036.
35. Taftachi F., Sanaei-Zadeh H., Sepehrian B. and Zamani N. Lipid emulsion improves Glasgow coma scale and decreases blood glucose level in the setting of acute non-local anesthetic drug poisoning--a randomized controlled trial. *Eur Rev Med Pharmacol Sci*. 2012; 16 Suppl 1:38-42. PMID: 22582483.
36. Goharbari M.H., Taghaddosinejad F., Arefi M., Sharifzadeh M., Mojtahedzadeh M., Nikfar S., et al. Therapeutic effects of oral liothyronine on aluminum phosphide poisoning as an adjuvant therapy: A clinical trial. *Hum Exp Toxicol*. 2018; 37(2):107-117. https://doi: 10.1177/0960327117694074. PMID: 29233028.
37. Shaker H.O., Rageh O.E., Alnajar M., Alshamaly N.F., Abdelmaged W.A. and Abd-ElGawad M. Efficacy of intravenous N acetylcysteine as an adjuvant therapy in the treatment of acute aluminum phosphide Poisoning: a systematic review and meta-analysis. *BMC Pharmacology and Toxicology*. 2023; 24:59. https://doi.org/10.1186/s40360-023-00699-2.

38. Hussien S.A., Al Teftazany E.A., Hagras A.M., Elhelaly H. and Abdelhamed W.G. N-acetylcysteine Use in Treatment of Acute Aluminium Phosphide Poisoning: Systematic Review and Meta-Analysis. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology*. 2023; 41:50-62. https://doi.org/10.21608/AJFM.2023.310229.
39. Taghaddosinejad F., Farzaneh E., Ghazanfari-Nasrabad M., Eizadi-Mood N., Hajihosseini M. and Mehrpour O. The effect of N-acetyl cysteine (NAC) on aluminum phosphide poisoning inducing cardiovascular toxicity: a case-control study. *Springerplus*. 2016; 5(1):1948. https://doi: 10.1186/s40064-016-3630-2. PMID: 27917341; PMCID: PMC5102994.
40. Tehrani H., Halvaie Z., Shadnia S., Soltaninejad K. and Abdollahi M. Protective effects of N-acetylcysteine on aluminum phosphide-induced oxidative stress in acute human poisoning. *Clin Toxicol (Phila).* 2013; 51(1):23-8. https://doi: 10.3109/15563650.2012.743029.. PMID: 23148565.
41. El Shehaby D.M., Sayed S.A., Abd El-Kareem D.M., Elsherif R. and Almaz D. Trimetazedine with hyperinsulinimea-euoglycemia, N-acetyl cysteine, and vitamin C: A new approach concept for management of aluminum phosphide poisoning. *J Biochem Mol Toxicol.* 2022; Jan;36(1):e22931. https://doi: 10.1002/jbt.22931. Epub 2021 Oct 19. PMID: 34665895..
42. Darwish R.T., Sobh Z.K., Hamouda E.H. and Saleh E.M. The efficacy of Coenzyme Q10 and liquid paraffin oil in the management of acute aluminum phosphide poisoning. *Toxicol Res (Camb).* 2020; 9(4):444-453. https://doi: 10.1093/toxres/tfaa045. PMID: 33936588; PMCID: PMC8059132.
43. Jemal K., Sandeep B. and Pola S. Phytochemical screening and in vitro antioxidant activity analysis of leaf and callus extracts of Allophylus serratus (ROXB) KURZ. *Jordan j. pharm. sci.* 2022; 15(1):51-69. https://jjournals.ju.edu.jo/index.php/jjps/article/view/291
44. Karki D., Pandey B., Jha P., Acharya A., Khanal D.P., Raut B. and Panthi S. Senna alata: Phytochemistry, Antioxidant, Thrombolytic, Anti-inflammatory, Cytotoxicity, Antibacterial activity, and GC-MS analysis. *Jordan j. pharm. sci*. 2024; 17(3):549-66. https://jjournals.ju.edu.jo/index.php/jjps/article/view/2406.

هل يمكن لمستحلب الدهون الوريدي أن يقلل معدل الوفيات في حالات التسمم الحاد بفوسفيد الألومنيوم؟ مراجعة منهجية

هويدا سعيد محمد[1]، رحمة محمود سعد[2]، أحمد ثروت النمروتي[3]، ريم إيهاب فاروق[4]، سمر رمضان محمد عبد الغني[1]، حنان الإمام[5]

[1] قسم الطب الشرعى والسموم الإكلينيكية، كلية الطب، جامعة القاهرة، مصر

[2] كلية الطب، الجامعة الحديثة للتكنولوجيا والمعلومات، مصر

[3] كلية الطب، جامعة الجلالة، مصر

[4] الصيدلة الإكلينيكية، كلية الصيدلة، جامعة القاهرة، مصر

[5] الكيمياء الحيوية، كلية الصيدلة، جامعة السادات، مصر

ملخص

الخلفية: يعتبر التسمم الحاد بالمبيدات الحشرية مشكلة صحية عامة وخطيرة منذ عقود. يتم علاجه بشكل رئيسي من خلال الأساليب الداعمة. يوفر علاج الإنقاذ بمستحلب الدهون الوريدي استراتيجية محتملة أخرى للعلاج. تهدف هذه الدراسة إلى تقديم مراجعة منهجية للأدبيات حول دور مستحلب الدهون الوريدي في علاج سمية فوسفيد الألومنيوم.

الطرق: تم إجراء بحث إلكتروني في قواعد البيانات التالية: بيميد، سجل كوكرين للتجارب ذات الشواهد، مراجعات كامبل، سكوبس، شبكة العلوم، سبرنجر للطبيعة، إلسفير، باحث جوجل العلمي، مجلات المنصورة، مجلات الزقازيق، مجلات عين شمس، ومجلات هندية.

النتائج: حققت 5 دراسات معايير الاشتمال، كان إجمالي حجم العينة 224 مريضًا، تلقى 102 مريضًا مستحلب الدهن الوريدي، وتم استخدام مستحلب الدهن الوريدي 20٪ في جميع الدراسات؛ استخدمته ثلاث دراسات بجرعة 10 مل/ساعة بالتسريب الوريدي. بينما استخدمته دراستان أخرتان بجرعة 1، 2، 3 مل/كجم و1.5 مل/كجم على مدى دقيقة واحدة يتبعها تسريب مستمر. وكان معدل الوفيات الإجمالي 68.6٪ في مجموعة مستحلب الدهن الوريدي و76.2٪ في المجموعة الضابطة. كان وقت البقاء على قيد الحياة وخروج الناجين أقصر في مجموعة مستحلب الدهون الوريدي.

الاستنتاج: مستحلب الدهون في الوريد هو نهج جديد في علم السموم، ويمكن أن يقلل من معدل الوفيات، ووقت المستشفى وخروج الناجين.

الكلمات الدالة: فوسفيد، ألومنيوم، وريدي، مستحلب، علاج.

* ***المؤلف المراسل: هويدا سعيد محمد***

mhowaida51@kasralainy.edu.eg

تاريخ استلام البحث 2024/02/04 وتاريخ قبوله للنشر 2024/06/13.

Virtual Screening for Molecular Targets of Emodin Against Red Complex Pathogens

Sriraj Gannamaneni[1], Anitha P[1], Smiline Girija A.S[2], Vijayashree Priyadharsini J[1*]

[1]Clinical Genetics Lab, Centre for Cellular and Molecular Research, Saveetha Dental College & Hospital, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai, India.
[2]Department of Microbiology, Centre for Infectious Diseases, Saveetha Dental College & Hospital, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai, India.

ABSTRACT

Objective: Periodontitis is a chronic inflammatory disease affecting teeth' supporting tissues. It is caused by specific bacterial species, including *Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf),* and *Treponema denticola,* known as the "red complex" group. These bacteria manipulate the immune response and promote tissue destruction, making them key players in periodontal pathogenesis. The present study aims to identify the potential molecular targets of Emodin against the red complex pathogens.

Method: The interaction between the phytocompound Emodin and red complex pathogens was identified using the STITCH tool. The proteins identified were then classified into functional categories using the VICMPred. The virulent proteins identified were then subjected to Bepired prediction, which provided information about the epitopes in the virulent proteins. Finally, the subcellular location of the proteins was demonstrated with the pSORTb tool.

Results: Carbamoyl-phosphate synthase is a large subunit identified as a virulence protein in *Pg* and *Tf*. DNA topoisomerase IV subunit A was found to be the common virulence protein for *Pg* and *Td.* The DNA gyrase subunit A and ATPase/histidine kinase/DNA gyrase B/HSP90 domain-containing protein were found to be identified in *Td* and *Tf.* It was the only protein predicted to be in the cytoplasmic membrane, while others were found in the cytoplasm. The four virulent proteins targeted by Emodin were found to harbor multiple epitopes.

Conclusion: Emodin was found to interact with all three pathogens of the red complex group. However, further experimental validation is warranted to prove the antimicrobial effect of Emodin against periodontal pathogens.

Keywords: Emodin, Red complex pathogens, Epitopes, computational tools, virulent protein.

1. INTRODUCTION

Periodontitis is a disease caused by a specific microbial composition found on the surface of teeth and tooth roots. Still, bacteria's exact contribution to the disease's progression is not yet fully understood. Commensal bacteria are believed to be protective in preventing disease development. However, some bacterial species, including *Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola,* found in plaque, use various mechanisms to interfere with host defense mechanisms, leading to disease progression [1]. *Porphyromonas gingivalis (Pg),* a Gram-negative oral anaerobe, is a pivotal player in periodontitis pathogenesis among over 500 oral bacterial species. While a natural member of the oral microbiome, its specialized virulence factors enable destructive proliferation in periodontal lesions, marking it as a pathobiont. Certain spiral-shaped bacteria species, particularly *Treponema denticola (Td),* are considered significant players in the development and progression of

***Corresponding author: Vijayashree Priyadharsini J**
vijayashreej.sdc@saveetha.com
Received: 06/02/2024 Accepted: 14/06/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2347

 © 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

periodontal disease [3]. *Tannerella forsythia (Tf)* is a Gram-negative oral pathogen that targets multiple proteins associated with virulence via its O-glycosylation system [4]. The emerging phenotype of multi-drug resistance exhibited by these pathogens underscores the need for alternative therapy, emphasizing herbal medicines. Identifying potential targets of a phyto compound against oral pathogens will provide insights into the compound's mode of action and aid in predicting the application of the compound either individually or in combination with other therapeutic modalities.

Emodin is a natural anthraquinone derivative found in various plants, fungi, and lichens. It has a rich history in traditional Chinese medicine and has been found to exhibit a range of biological activities. These include antibacterial, anti-inflammatory, and anticancer properties. It was found to show promising effects in reversing chemotherapy resistance and has antimalarial and antiallergic effects [5]. A recent study examined the photodynamic effects of aloe-emodin (AE) on *Candida albicans*, a common fungus that can be resistant to some medications. The researchers used antimicrobial photodynamic therapy (PDT) to test the effectiveness of AE, a natural compound found in *Aloe vera* and *Rheum palmatum*. The results showed that AE had no negative effects in the absence of light but effectively eliminated *C. albicans* cells *in vitro* when exposed to light. Confocal microscopy also revealed that fungal cells took up AE more easily when exposed to light. Finally, transmission and scanning electron microscopy showed that AE-mediated aPDT caused damage to the cell structures of *C. albicans*, suggesting that AE could potentially be used as a photosensitizer to combat drug-resistant strains of the fungus [6]. Although, several plant compounds have been assessed using *in vitro* approaches to deduce their biological activities [7], it has been a time-consuming and expensive process demanding labor and expertise in the field. Alternatively, computational methods have been considered to be a boon to researchers, wherein the initial screening of multiple bioactive compounds can be done within less time duration and an inexpensive manner [8]. In line with this, the phytocompound emodin was assessed for its interaction with a red complex pathogen to develop therapeutic strategies employing Emodin empoying computational tools to elucidate their activities against periodontal pathogens.

2. MATERIALS AND METHOD

Strains and phytocompound used in the study

The phytocompound Emodin was tested against red complex pathogens, namely *Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia*. The STITCH tool [9] revealed the interaction between the compound and the pathogen's protein repertoire.

Analyzing protein Interaction Network

STITCH (Search tool for interactions of chemicals) is an exhaustive pipeline that can be used to predict the interaction between chemicals and proteins. The interaction is of two types: (a) direct or physical (b) indirect or functional association, which arises from data accumulated in the primary databases. The repertoire of proteins against red complex pathogens, namely *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythia* interacting with Emodin, were used for predicting virulence [9]. The FASTA format sequences were retrieved from the National Centre of Biotechnology information domain and used for predicting the functional class of proteins and their virulence properties (https://www.ncbi.nlm.nih.gov/protein/?term=).

Prediction of functional class of interacting protein

VICMpred server classifies the protein identified into four major classes: virulence factor, information and storage processing, cellular process, and metabolism. Anchorage-dependent protein effluent pumps, transporters, toxins, and hemolytic molecules are identified based on the support vector machine (SVM) algorithm, which classifies proteins based on their amino acid composition pattern [10].

Prediction of B cell Epitope in the virulence proteins

BepiPred is a software tool that predicts linear B-cell epitopes in protein sequences. It uses machine learning

algorithms that have been trained on known epitope data to identify protein regions that are probably recognized by antibodies. Researchers can apply BepiPred to facilitate the identification of possible antigenic sites on proteins, which is useful for vaccine development, immunotherapy, and understanding immune responses. This tool helps analyze antigen-antibody interactions and design experiments related to antibody production and detection. Epitopes are antigen-determining sites on the virulent proteins capable of eliciting an immune response in the host. Identifying those B cell epitopes on virulence protein adds merit to the compound. Bepred on the virulent proteins. The peptide molecules that score above a threshold >0.5 are predicted to be part of the epitope and are colored yellow in the graph [11, 12].

Prediction of subcellular localization of protein

pSORTb is a computational tool that predicts bacterial proteins' subcellular localization (SCL). Its main goal is to determine where proteins are located within bacterial cells, which helps researchers understand protein function and cellular processes better. pSORTb uses various protein features such as amino acid composition, sequence motifs, and similarity to known proteins to make accurate predictions. Using this tool, researchers can identify potential drug targets or vaccine candidates by studying bacterial proteomes. Cell surface proteins are readily targeted, while the cytoplasmic or nuclear protein needs a proper drug delivery system to target the protein of interest. Hence, pSORTb was used for the identification of sub-cellular locations of virulence protein (PSORTdb 4.0 (http://db.psort.org/)) [13].

3. RESULTS

Emodin was found to interact with the red complex pathogens *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythia.* Metabolism-related proteins were found to predominate in *Pg, Tf, a*nd *Td.* The DNA topoisomerase IV subunit A and carbamoyl-phosphate synthase large subunit were found to be the virulence factors in the *Pg* group (Table 1a, Figure 1a), DNA topoisomerase IV subunit B and DNA gyrase subunit A were found to be the confer virulence in *Td* (Table 1b, Figure 1b), while ATPase/histidine kinase/DNA gyrase B/HSP90 domain-containing protein and Carbamoyl-phosphate synthase large subunit were found to be the virulent proteins in *Tf* (Table 1c, Figure 1c). All the virulent proteins except for ATPase/histidine kinase/DNA gyrase B/HSP90 domain-containing protein were found to be localized in the cytoplasm. The prediction of epitopes in the virulent proteins demonstrated that these proteins were composed of multiple epitopes (Figure 2).

Table 1a: The list of proteins of *Porphyromonas gingivalis* interacting with emodin

Organism	Identifier	Protein	VICMPred
Porphyromonas gingivalis	**PGN_0472**	**DNA topoisomerase IV subunit A**	**Virulence factors**
	PGN_1594	DNA topoisomerase IV subunit B	Cellular Process
	PGN_2019	Bifunctional UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase/(3R)-hydroxymyristoyl-ACP dehydratase	Metabolism molecule
	PGN_1449	Inosine 5-monophosphate dehydrogenase	Metabolism molecule
	PGN_1443	**Carbamoyl-phosphate synthase large subunit**	**Virulence factors**
	gyrA	DNA gyrase A subunit	Metabolism molecule
	gyrB	DNA gyrase B subunit	Metabolism molecule
	pyrB	Aspartate carbamoyltransferase	Metabolism molecule
	carA	Carbamoyl phosphate synthase small subunit	Cellular Process
	hisS	Histidyl-tRNA synthetase	Information and Storage

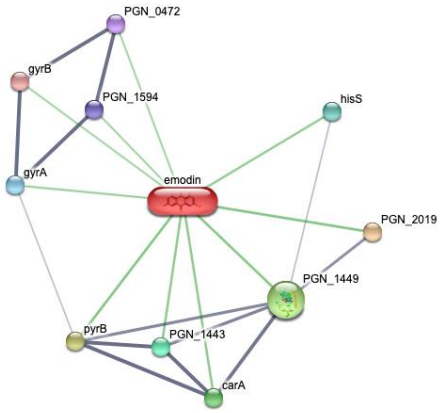


Figure 1a: The protein interaction network of *Porphyromonas gingivalis* with emodin

Table 1b: The list of proteins of *Treponema denticola* interacting with emodin

Organism	Identifier	Protein	VICMPred
Treponema denticola	**TDE_2245**	**DNA topoisomerase IV subunit B**	**Virulence Factors**
	TDE_0492	Sensor histidine kinase/response regulator	Metabolism Molecule
	TDE_2693	Ankyrin repeat-containing protein	Metabolism Molecule
	TDE_0502	Ankyrin repeat-containing protein	Metabolism Molecule
	TDE_0823	(3R)-hydroxymyristoyl-ACP dehydratase	Cellular Process
	TDE_2450	Ankyrin repeat-containing protein	Metabolism Molecule
	gyrA	**DNA gyrase subunit A**	**Virulence Factors**
	pyrBI	Bifunctional aspartate carbamoyltransferase catalytic subunit/aspartate carbamoyltransferase regulatory subunit	Cellular Process
	guaB	Inosine 5-monophosphate dehydrogenase	Cellular Process
	hisS	Histidyl-tRNA synthetase	Metabolism Molecule

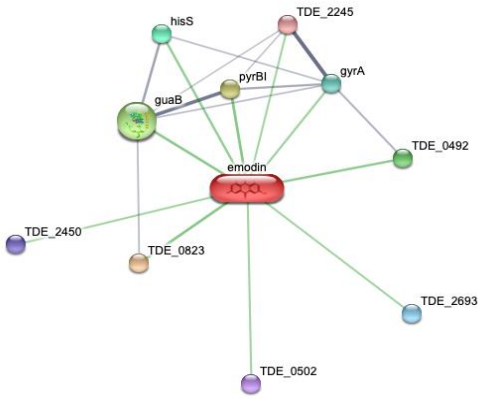


Figure 1b: The protein interaction network of *Treponema denticola* with emodin

Table 1c: The list of proteins of *Tannerella forsythia* interacting with emodin

Organism	Identifier	Protein	VICMPred
Tannerella forsythia	BFO_1082	Putative DNA gyrase, B subunit	Metabolism molecule
	BFO_2044	Putative inosine-5'-monophosphate dehydrogenase	Metabolism molecule
	BFO_2981	Kinase domain-containing protein	Metabolism molecule
	BFO_2007	**ATPase/histidine kinase/DNA gyrase B/HSP90 domain-containing protein**	**Virulence factors**
	gyrA	DNA gyrase subunit A	Cellular Process
	gyrB	DNA gyrase subunit B	Metabolism molecule
	hisS	Histidine--tRNA ligase	Metabolism molecule
	fabZ	Beta-hydroxyacyl-(acyl-carrier-protein) dehydratase FabZ	Cellular Process
	pyrB	Aspartate carbamoyltransferase	Metabolism molecule
	carB	**Carbamoyl-phosphate synthase large subunit**	**Virulence factors**

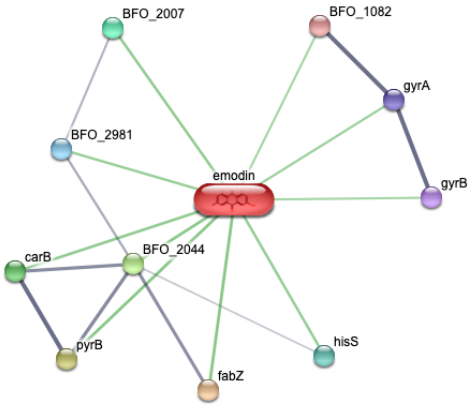


Figure 1c: The protein interaction network of *Tannerella forsythia* with emodin

Table 2: Subcellular localization of virulent proteins identified using VICMPred

Identifier	Virulent Protein	Subcellular localization	Score
PGN_0472	DNA topoisomerase IV subunit A	Cytoplasmic	9.97
PGN_1443	Carbamoyl-phosphate synthase large subunit	Cytoplasmic	9.94
TDE_2245	DNA topoisomerase IV subunit B	Cytoplasmic	9.97
gyrA	DNA gyrase subunit A	Cytoplasmic	9.97
BFO_2007	ATPase/histidine kinase/DNA gyrase B/HSP90 domain-containing protein	Cytoplasmic membrane	7.88
carB	Carbamoyl-phosphate synthase large subunit	Cytoplasmic	9.97

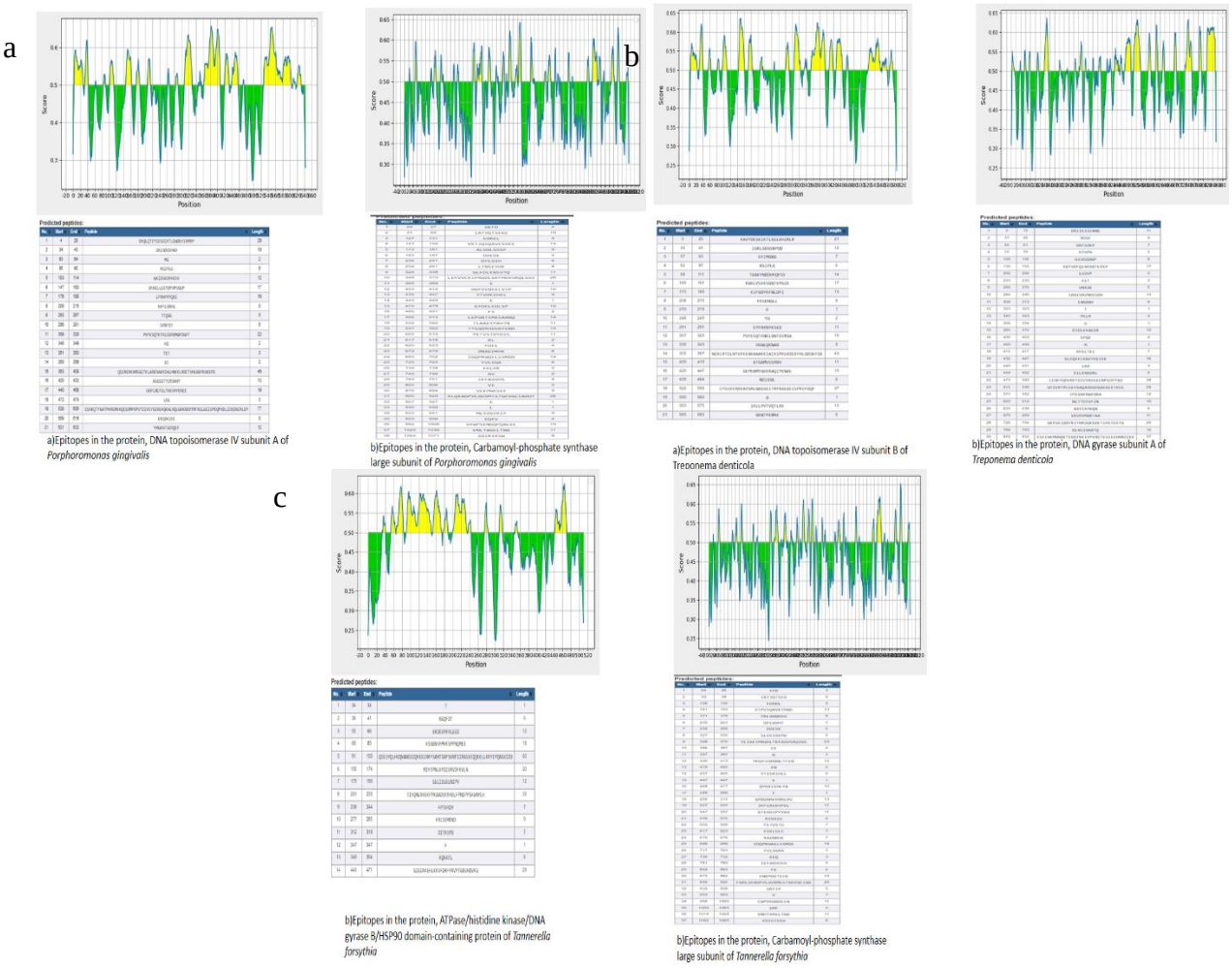


Figure 2: Epitopes in the virulent proteins of red complex pathogen a) *Porphoromonas gingivalis* b) *Treponema denticola* c) *Tannerella forsythia* targeted by Emodin

4. DISCUSSION

Emodin was found to target the virulent proteins of the red complex pathogens, which is evident from the STITCH and VICMPred predictions. Furthermore, the subcellular localization demonstrated that most proteins were found in the cytoplasm. The presence of multiple epitopes in the virulent protein can facilitate further analysis of Emodin and the proteins using molecular docking studies. A study investigated the relationship between the count of red-complex bacteria in saliva, specifically *Porphyromonas gingivalis*, and the periodontal status in a Japanese population. The study utilized real-time polymerase chain reaction analysis and clinical assessments to determine that *P. gingivalis* levels strongly correlated with periodontal parameters such as probing pocket depth, bleeding on probing, and bone crest level. The detection of *P. gingivalis* and *Treponema denticola* and/or Tannerella forsythia in the saliva indicated a more severe periodontal condition, highlighting the importance of *P. gingivalis* in the progression of periodontitis [14]. A study presents novel aloe emodin-hybridized sulfonamide aminophosphates as potential antibacterial agents to combat drug-resistant bacterial infections. Among them, ethyl aminophosphate-hybridized sulfadiazine aloe emodin 7a (EASA-7a) exhibited potent antibacterial activity against drug-resistant *E. faecalis*, low hemolytic activity, and biofilm-disruptive ability. Mechanistic studies suggest its efficacy through membrane permeation, depolarization, ROS accumulation, and DNA intercalation. EASA-7a shows promise for further development in addressing life-threatening bacterial infections [15]. A study explored the possibility of using emodin, found in *Polygonum cuspidatum*, as a treatment for Glässer's disease, which affects swine and is caused by *Haemophilus parasuis*. The study found that emodin had strong inhibitory effects against *H. parasuis*, with minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of 32 and 64 μg/mL, respectively. Based on antibacterial kinetics, the activity of emodin was found to be concentration-dependent. Membrane integrity assays revealed that emodin can disrupt cell membranes and alter membrane protein conformation. Transmission electron microscopy showed significant cellular damage in *H. parasuis* treated with emodin, indicating its potential as a therapy for Glässer's disease [16].

A research study has identified a new class of haloemodins derived from traditional Chinese medicine that have promising potential as antibacterial agents against drug-resistant infections. These haloemodins have been found to inhibit bacterial topoisomerases while leaving human topoisomerases unaffected. They have shown remarkable efficacy against Gram-positive bacteria, including strains resistant to common antibiotics, such as vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus*. Furthermore, the antibacterial spectrum of haloemodins against Gram-negatives was expanded using polymyxin B nonapeptide. *In vivo* studies have confirmed that haloemodins have therapeutic efficacy in treating *S. aureus*-induced keratitis in rabbits, indicating their potential use as a promising antibacterial agent against drug-resistant pathogens [17]. Li and team investigated the efficacy of antimicrobial photodynamic therapy (aPDT) that uses aloe-emodin (AE) against multidrug-resistant (MDR) *Acinetobacter baumannii* clinical isolates. The study revealed that AE did not have any dark toxicity and could effectively inactivate MDR *A. baumannii* in a concentration and light-dose-dependent manner. The study also found that AE could damage bacterial components, including genomic DNA, membrane integrity, and cellular structure. These results indicate that AE could be a potential photosensitizer for treating superficial infections caused by MDR *A. baumannii* [18]. These reports clearly agreed with the observations made in the present study.

A study presented information regarding the design, synthesis, and evaluation of twenty aloe-emodin derivatives, focusing on their biological activities. Certain derivatives, particularly those with thiosemicarbazide moieties, displayed potent tyrosinase inhibition. Structure-activity relationships were discussed, and the inhibition mechanisms of selected

compounds were investigated. Additionally, some derivatives' antibacterial and anti-inflammatory activities were screened, revealing promising candidates for further exploration in drug development [19]. In addition to exhibiting anti-bacterial activity, Emodin was found to exhibit antiviral properties, which have been elucidated by researchers worldwide. In this context, a study explored modifying emodin's structure to enhance its activity against HCoV-NL63. Halogenation improved antiviral potency, with the iodinated analog E_3I exhibiting activity comparable to remdesivir, albeit with some observed toxicity to Vero cells [20]. Aloe-emodin is a potential inducer of interferon (IFN) with low levels of toxicity. It activates the IFN-stimulated response element (ISRE) and the gamma-activated sequence (GAS)-driven cis-reporting systems, which upregulates IFN-stimulated genes. Aloe-emodin also significantly enhances nitric oxide production and has demonstrated antiviral activity against Japanese encephalitis virus (JEV) and enterovirus 71 (EV71) in a dose- and time-dependent manner. This suggests that it has potential through IFN signaling responses [21]. A recent study by Luo and the team demonstrated Aloe-emodin's (Ae) antiviral activity against the African swine fever virus (ASFV). Studies have shown that Ae can inhibit the replication of ASFV (African Swine Fever Virus) by reducing the activation of the NF-κB signaling pathway, which ASFV induces. Ae (emodin) also induced apoptosis (cell death) in infected cells by modulating the expression of apoptotic proteins. These findings offer potential therapeutic strategies for preventing and treating ASF, highlighting how Ae can help combat viral infections [22].

Numerous *in silico* and *in vitro* studies have been conducted with crude extracts or bioactive compounds extracted from plants. These studies reiterate using phytocompounds as potential antimicrobial leads against different classes of microorganisms [23, 24]. Molecular docking [25] and network pharmacology [26] have aided in demonstrating the mechanism of action of plant compounds and their mode of action against pathogens. Thus, the study design using computational approaches has reduced the cost and time required for analyzing bioactive compounds and paved the way for identifying novel drug targets or therapeutic leads.

Limitations: Computational methods have been utilized in the field of drug discovery and development for a long time. These in silico approaches have several advantages, such as cost-effectiveness, reduced investigation time, and real-time application of preliminary results. However, this approach has some limitations, including the need to validate data in a biological system to confirm findings. Additionally, interactions observed may be purely physical or have functional consequences that require further exploration. Furthermore, certain proteins found in bacteria may share close homology with host proteins, and in vivo experiments should be conducted to rule out any adverse side effects anticipated during the therapeutic usage of the drug.

5. CONCLUSION

In summary, this virtual investigation potentially reveals valuable insights into the molecular targets of Emodin in red complex pathogens. These findings are important for developing targeted treatments for dental caries by shedding light on the complex molecular interactions between Emodin and these microorganisms that cause periodontitis. However, further research and refinement are needed to fully utilize Emodin as a therapeutic agent against these red complex pathogens.

Declaration of competing interest: The authors have no conflicts of interest relevant to this article.

Financial & competing Interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

REFERENCES

1. Darveau R.P. Periodontitis: a polymicrobial disruption of host homeostasis. *Nat Rev Microbiol*. 2010; 8(7):481-90. doi: 10.1038/nrmicro2337.
2. Mysak J., Podzimek S., Sommerova P., Lyuya-Mi Y., Bartova J., Janatova T., Prochazkova J. and Duskova J. Porphyromonas gingivalis: major periodontopathic pathogen overview. *J Immunol Res.* 2014; 2014:476068. doi: 10.1155/2014/476068.
3. Zeng H., Chan Y., Gao W., Leung W.K. and Watt R.M. Diversity of Treponema denticola and Other Oral Treponeme Lineages in Subjects with Periodontitis and Gingivitis. *Microbiol Spectr*. 2021; 9(2):e0070121. doi: 10.1128/Spectrum.00701-21.
4. Veith P.D., Scott N.E. and Reynolds E.C. Characterization of the O-Glycoproteome of Tannerella forsythia. *mSphere*. 2021; 6(5):e0064921. doi: 10.1128/mSphere.00649-21.
5. Stompor-Gorący M. The Health Benefits of Emodin, a Natural Anthraquinone Derived from Rhubarb-A Summary Update. *Int J Mol Sci*. 2021; 22(17):9522. doi: 10.3390/ijms22179522.
6. Ma W., Liu C., Li J., Hao M., Ji Y. and Zeng X. The effects of aloe emodin-mediated antimicrobial photodynamic therapy on drug-sensitive and resistant Candida albicans. *Photochem Photobiol Sci*. 2020; 19(4):485-494. doi: 10.1039/c9pp00352e.
7. Abu-Darwish D., Shibli R. and Al-Abdallat A.M. Phenolic Compounds and Antioxidant Activity of *Chiliadenus montanus* (Vhal.) *Brullo* grown in vitro. *Jordan Journal of Pharmaceutical Sciences.* 2024; 17(3):611–628. doi: 10.35516/jjps.v17i3.2248.
8. Sowndhariya S.S., Ravi S., Dharani J.D. and Sripathi R.S. Chemical Constitution, In-silico Molecular Docking Studies and Antibacterial Activity of Flower Essential Oil of Artabotrys hexapetalus. *Jordan Journal of Pharmaceutical Sciences.* 2022; 15(3):341–354. doi: 10.35516/jjps.v15i3.408.
9. Szklarczyk D., Santos A., von Mering C., Jensen L.J., Bork P. and Kuhn M. STITCH 5: augmenting protein-chemical interaction networks with tissue and affinity data. *Nucleic Acids Res.* 2016; 44(D1): D380-4. doi: 10.1093/nar/gkv1277.
10. Saha S. and Raghava G.P. VICMpred: an SVM-based method for the prediction of functional proteins of Gram-negative bacteria using amino acid patterns and composition. *Genomics Proteomics Bioinformatics.* 2006; 4(1):42-7. doi: 10.1016/S1672-0229(06)60015-6.
11. Larsen J.E., Lund O. and Nielsen M. Improved method for predicting linear B-cell epitopes. *Immunome Res.* 2006; 2:2. doi: 10.1186/1745-7580-2-2.
12. Jespersen M.C., Peters B., Nielsen M. and Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic Acids Res.* 2017; 45(W1):W24-W29. doi: 10.1093/nar/gkx346.
13. Lau W.Y.V., Hoad G.R., Jin V., Winsor G.L., Madyan A., Gray K.L., Laird M.R., Lo R. and Brinkman F.S.L. PSORTdb 4.0: expanded and redesigned bacterial and archaeal protein subcellular localization database incorporating new secondary localizations. *Nucleic Acids Res.* 2021; 49(D1): D803-D808. doi: 10.1093/nar/gkaa1095.
14. Chigasaki O., Aoyama N., Sasaki Y., Takeuchi Y., Mizutani K., Ikeda Y., Gokyu M., Umeda M., Izumi Y., Iwata T. and Aoki A. Porphyromonas gingivalis, the most influential pathogen in red-complex bacteria: A cross-sectional study on the relationship between bacterial count and clinical periodontal status in Japan. *J Periodontol.* 2021; 92(12):1719-1729. doi: 10.1002/JPER.21-0011.
15. Deng Z., Sun H., Bheemanaboina R.R.Y., Luo Y. and Zhou C.H. Natural aloe emodin-hybridized sulfonamide aminophosphates as novel potential membrane-perturbing and DNA-intercalating agents against Enterococcus faecalis. *Bioorg Med Chem Lett.* 2022; 64:128695. doi: 10.1016/j.bmcl.2022.128695.

16. Li L., Song X., Yin Z., Jia R., Li Z., Zhou X., Zou Y., Li L., Yin L., Yue G., Ye G., Lv C., Shi W. and Fu Y. The antibacterial activity and action mechanism of emodin from Polygonum cuspidatum against Haemophilus parasuis in vitro. *Microbiol Res.* 2016; 186-187:139-45. doi: 10.1016/j.micres.2016.03.008.
17. Duan F., Li X., Cai S., Xin G., Wang Y., Du D., He S., Huang B., Guo X., Zhao H., Zhang R., Ma L., Liu Y., Du Q., Wei Z., Xing Z., Liang Y., Wu X., Fan C., Ji C., Zeng D., Chen Q., He Y., Liu X. and Huang W. Haloemodin as novel antibacterial agent inhibiting DNA gyrase and bacterial topoisomerase I. *J Med Chem.* 2014; 57(9):3707-14. doi: 10.1021/jm401685f.
18. Li J., Qin M., Liu C., Ma W., Zeng X. and Ji Y. Antimicrobial photodynamic therapy against multidrug-resistant Acinetobacter baumannii clinical isolates mediated by aloe-emodin: An in vitro study. *Photodiagnosis Photodyn Ther*. 2020; 29:101632. doi: 10.1016/j.pdpdt.2019.101632.
19. Liu J., Wu F. and Chen C. Design and synthesis of aloe-emodin derivatives as potent anti-tyrosinase, antibacterial and anti-inflammatory agents. *Bioorg Med Chem Lett.* 2015; 25(22):5142-6. doi: 10.1016/j.bmcl.2015.10.004.
20. Horvat M., Avbelj M., Durán-Alonso M.B., Banjanac M., Petković H. and Iskra J. Antiviral Activities of Halogenated Emodin Derivatives against Human Coronavirus NL63. *Molecules.* 2021; 26(22):6825. doi: 10.3390/molecules26226825.
21. Lin C.W., Wu C.F., Hsiao N.W., Chang C.Y., Li S.W., Wan L., Lin Y.J. and Lin W.Y. Aloe-emodin is an interferon-inducing agent with antiviral activity against Japanese encephalitis virus and enterovirus 71. *Int J Antimicrob Agents.* 2008; 32(4):355-9. doi: 10.1016/j.ijantimicag.2008.04.018.
22. Luo Y., Yang Y., Wang W., Gao Q., Gong T., Feng Y., Wu D., Zheng X., Zhang G. and Wang H. Aloe-emodin inhibits African swine fever virus replication by promoting apoptosis via regulating NF-κB signaling pathway. *Virol J.* 2023; 20(1):158. doi: 10.1186/s12985-023-02126-8.
23. Widyananda M.H., Kurniasari C.A., Alam F.M., Rizky W.C., Dings T.G.A., Ansori A.N.M. and Antonius Y. Exploration of Potentially Bioactive Compounds from Fingerroot (*Boesenbergia rotunda* L.) as Inhibitor of Atherosclerosis-Related Proteins (CETP, ACAT1, OSC, sPLA2): An in silico Study. *Jordan Journal of Pharmaceutical Sciences.* 2023; 16(3):550–564. doi: 10.35516/jjps.v16i3.1609.
24. Girija A.S.S, Gunasekaran S., Habib S., Aljeldah M., Al Shammari B.R., Alshehri A.A., Alwashmi A.S.S., Turkistani S.A., Alawfi A., Alshengeti A., Garout M., Alwarthan S., Alsubki R.A., Moustafa N.M. and Rabaan A.A. Prediction of Putative Epitope Peptides against BaeR Associated with TCS Adaptation in Acinetobacter baumannii Using an In Silico Approach. *Medicina (Kaunas).* 2023; 59(2):343. doi: 10.3390/medicina59020343.
25. Uma Maheswari K. and Sankar S. In Silico Molecular Docking of Phytochemicals of Murraya koenigii Against Streptococcus mutans. *Cureus*. 2024; 16(2):e53679. doi:10.7759/cureus.53679
26. Suresh M., Sai K.V., Mitra K., Ravindran R. and Doble M. A network pharmacology-based approach to understanding the mechanism of action of anti-mycobacterial activity of Acacia nilotica: a modeling and experimental study. *Mol Divers*. doi:10.1007/s11030-024-10985-8

الفحص الافتراضي للأهداف الجزيئية لمادة الإمودين ضد مسببات الأمراض المعقدة الحمراء

سيراج غانامانيني[1]، أنيثا بي[1]، سميلين جيريجا أ.س[2]، فيجياشري بريادارسيني ج[1]*

[1] مختبر الوراثة السريرية، مركز الأبحاث الخلوية والجزيئية، كلية ومستشفى سافيثا لطب الأسنان، معهد سافيثا للعلوم الطبية والتقنية [SIMATS]، جامعة سافيثا، تشيناي، الهند.

[2] قسم علم الأحياء الدقيقة، مركز الأمراض المعدية، كلية ومستشفى سافيثا لطب الأسنان، معهد سافيثا للعلوم الطبية والتقنية [SIMATS]، جامعة سافيثا، تشيناي، الهند.

ملخص

الهدف: التهاب دواعم السن هو مرض التهابي مزمن يصيب الأنسجة الداعمة للأسنان. يحدث بسبب أنواع بكتيرية معينة، بما في ذلك (Pg) *Porphyromonas gingivalis*، و *Tannerella forsythia* (Tf)، و *Treponema denticola*)، والمعروفة بمجموعة "المركب الأحمر ."تستغل هذه البكتيريا الاستجابة المناعية وتعزز تدمير الأنسجة، مما يجعلها عناصر أساسية في مسببات أمراض دواعم السن. تهدف الدراسة الحالية إلى تحديد الأهداف الجزيئية المحتملة لمركب الإيمودين ضد مسببات الأمراض من مجموعة المركب الأحمر.

الطريقة: تم تحديد التفاعل بين المركب النباتي إيمودين ومسببات الأمراض من مجموعة المركب الأحمر باستخدام أداة .STITCHثم تم تصنيف البروتينات التي تم تحديدها إلى فئات وظيفية باستخدام أداة .VICMPred وبعد ذلك، تم إخضاع البروتينات الفيروسية لتوقعات Bepired، التي قدمت معلومات حول الحواتم في البروتينات الفيروسية. وأخيراً، تم تحديد الموقع داخل الخلية للبروتينات باستخدام أداة .pSORTb

النتائج: تم تحديد سينثاز كارباموبل فوسفات كوحدة فرعية كبيرة كأحد البروتينات الفيروسية في Pgو.Tf كما وُجد أن وحدة DNA topoisomerase IV الفرعية A هي البروتين الفيروسي المشترك بين Pgو.Td وُجد أن وحدة DNA gyrase الفرعية A والبروتين المحتوي على نطاقات /ATPaseكيناز الهستيدين/ DNA gyrase B/HSP90 تم تحديدهما في Td و.Tf كان هذا البروتين الوحيد المتوقع أن يكون في غشاء السيتوبلازم، بينما وُجدت البروتينات الأخرى في السيتوبلازم. وُجد أن البروتينات الفيروسية الأربعة المستهدفة بواسطة إيمودين تحتوي على حواتم متعددة.

الخلاصة: وُجد أن الإيمودين يتفاعل مع جميع مسببات الأمراض الثلاثة من مجموعة المركب الأحمر. ومع ذلك، هناك حاجة لمزيد من التجارب لإثبات التأثير المضاد للميكروبات للإيمودين ضد مسببات أمراض دواعم السن.

الكلمات الدالة: الإيمودين، مسببات الأمراض المعقدة الحمراء، الحواتم، الأدوات الحسابية، البروتين الضار.

* ***المؤلف المراسل: فيجياشري بريادارسيني ج***

vijayashreej.sdc@saveetha.com

تاريخ استلام البحث 2024/02/06 وتاريخ قبوله للنشر 2024/06/14.

In Vivo Evaluation of Genotoxicity and Antioxidant Capacity of *Ajuga Orientalis* L. (Lamiaceae) Leaf Extracts

Mohammad M. Al-Gharaibeh[1*], Ahmed O. Maslat[2], Noor Alyaqin A. Kanakri[2], Yazun B. Jarrar[3]

[1]Department of Plant Production, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan.
[2]Department of Biology, Faculty of Science, Yarmouk University, Irbid, Jordan.
[3]Department of Basic Medical Sciences, Faculty of Medicine, Al-Balqa Applied University, Al-Salt, Jordan.

ABSTRACT

Ajuga orientalis L. (Lamiaceae) is a fragrant herb native to the Eastern Mediterranean region, widely used in traditional healing practices in Jordan and neighboring countries. Despite its extensive use, there is a lack of toxicological studies on its leaf extracts. This study aims to address this gap by evaluating the genotoxic potential of ethanolic and aqueous leaf extracts using a micronucleus (MN) assay on mice DNA, alongside assessing their antioxidant status. The median lethal dose 50% (LD_{50}) was tested in ten groups of sixty male *Balb/c* mice to determine the acute toxicity of *A. orientalis* leaf extracts. Four groups of male *Balb/c* mice (n=6) were used to evaluate micronucleus (MN) formation and total antioxidant capacity for each extract. Each group received daily intraperitoneal injections of one of the following concentrations: 4000, 2000, 1000, and 500 mg/kg over 28 days. Additionally, three control groups were included for comparison purposes. Peripheral blood samples were screened for MN formation, and liver samples were assessed for total antioxidant capacity. Results revealed an LD50 of 4000 mg/kg for both extracts, alongside a significant dose-dependent increase in MN formation and lower antioxidant capacity compared to controls. The findings indicate the genotoxicity of *A. orientalis* leaf extracts in *Balb/c* mice, urging caution in human consumption. Further research is warranted to comprehensively assess their safety and toxicity, especially considering their traditional medicinal use.

Keywords: medicinal plant; micronucleus; oriental bugle; total antioxidant capacity.

1. INTRODUCTION

The utilization of medicinal plant products and supplements has seen considerable growth in recent decades. Approximately 70-80% of the global population relies on traditional medicinal plants as a primary source of healthcare according to the World Health Organization (WHO). In spite of the accessibility of modern synthetic medications, individuals continue to opt for natural herbs for certain aspects of their primary healthcare [1]. Because they are natural, there is a prevalent perception that herbal products are inherently safer than allopathic medicines [2]. This perception in addition to low cost makes the use of herbal medicine more prominent. However, the use of herbal medicines without adherence to safety standards and without conducting toxicological studies is incorrect and harmful [3], potentially leading to adverse health effects due to their inherent toxic chemical composition [2].

Over the past few years, there has been increasing attention on investigating the toxicity, mutagenic properties, and potential carcinogenic effects of commonly used traditional medicinal plants [4]. The toxic effect of some medicinal plant extracts can lead to numerous chromosomal structure abnormalities, changes, and

[*]***Corresponding author: Mohammad M. Al-Gharaibeh***
mfagharaibeh@just.edu.jo
Received: 07/03/2024 Accepted: 14/06/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2466

 © 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

damage deoxyribonucleic acid (DNA) [5]. Such plants possessing DNA-damaging effects are classified as genotoxic plants [6]. Interestingly, it has been discovered that certain plants commonly used in traditional medicine, such as *Artemisia absinthium* L., *Crataegus oxyacantha* L. *Equisetum arvense* L., *Plantago lanceolata* L. and *Synadenium umbellatum* Pax, possess genotoxic potential [7–11]. DNA damage plays an etiological role in several human diseases including cancer [3]. The genotoxic potential was found not confined to a particular plant family, several studies have reported that proved genotoxic plants are common and frequent in the following families: *Fabaceae*, *Asteraceae*, *Euphorbiaceae*, *Rosaceae*, *Lamiaceae*, and *Apocynaceae* members [6]. Therefore, and for safety measures, a genotoxicity assessment of medicinal plants used in folkloric medicine appears to be required [8,12].

In Jordan, about 20% of the total flora are listed as medicinal plants [13]. Many of these plants have a long history of traditional use in medicine and are also utilized in the pharmaceutical industry [14]. While the biological effects and chemical composition of several herbal remedies used in traditional medicine have been investigated, many of them are still used without any validation of their safety and efficacy [1,15]. One of the most important and common herbal families worldwide including Jordan is the *Lamiaceae* family, members of this family genera such as *Salvia*, Teucrium, Thymus and Origanum showed wide medical and biological uses [16–18].

In Jordan and neighboring countries, there is an increasing demand for wild herbal plants, including *A. orientalis*, driven by the growing belief among local communities in their therapeutic properties, especially in cancer treatment. Moreover, the plant leaves are edible, either consumed fresh in salads or dried as a component of Jordanian manasaf spices. *Ajuga orientalis* L., commonly known as Eastern bugle, is a flowering species in the Lamiaceae family native to the eastern Mediterranean regions, including Jordan [19]. Due to its medicinal attributes, this species has found applications in traditional medicine across the globe for the treatment of conditions such as rheumatism, gout, malaria, and asthma, hypertension, hyperglycemia, joint pain, and gastrointestinal diseases [1,16,20–22]. Furthermore, members within the genus *Ajuga* have been documented for possessing medicinal properties such as: antibacterial, anti-inflammatory and antioxidant [23,24]. Notably, the ethanolic extract derived from the aerial parts of *A. orientalis* has exhibited noteworthy cytotoxic effects against breast and colon cancer [1]. In general, most cytotoxic plants are bears to have a genotoxic potential [2]. However, despite its widespread use and potential therapeutic benefits, there is a notable absence of comprehensive toxicological studies on *A. orientalis* leaf extracts. Our research aims to address this critical gap by evaluating the genotoxic potential and antioxidant status of these extracts. Assessing the safety profile and potential adverse effects of *A. orientalis* is crucial, especially considering the rising demand for this plant in traditional medicine.

2. MATERIALS AND METHODS

2.1. Plant material

The aerial parts of *A. orientalis*, at flowering period, were collected from roadside of Ishtafina forest, Ajloun city, north Jordan (32°35′47″N 35°76′85″E) in March 2021. The plant materials have been collected and authenticated by Dr. Mohammad Al-Gharaibeh (Department of Plant Production, J.U.S.T) and a voucher specimen (PHS-136) was deposited in the herbarium of the Faculty of Pharmacy, JUST.

2.2. Ethanolic and aqueous extracts preparation

Fresh leaves of *A. orientalis* were detached from stems, washed with tap water, and allowed to air-dry at room temperature in the shade for a period of three weeks. Afterward, they were finely ground into a powder. Ten grams of the powdered material were added to 100 ml of both solvents (distilled water and 90% ethanol) for extract preparation. For aqueous extract, the traditional way of

decoction preparation was followed by boiling the mixture over low heat for 5 minutes. Subsequently, the mixture was left to reflux for 30 minutes until it reached room temperature (approximately 25°C). Following this, the extract was centrifuged and filtered. For the ethanolic extract, the soaked powdered leaves with 90% ethanol were exhaustively extracted using the maceration technique for 24 h, with regular shaking. Subsequently, the extracts were centrifuged and filtered. To avoid the potential toxicity of ethanol, the extracts were diluted at a ratio of 1:5 (1 ml plant extract with a solution of 4 ml distilled water and 10% polyethylene glycol). The obtained crude extracts, both aqueous and ethanolic, were sealed in an airtight container and preserved at a temperature of -20°C for subsequent analysis. Different concentrations of the plant extracts were prepared by serial dilution.

2.3. Animal Study

Healthy 8-week-old male *Balb/c* mice with an average weight of 27.5 ± 3 g were employed to test the LD_{50}, MN formation, and total antioxidant capacity activity (TAC) of *A. orientalis* leaf extracts. The mice were housed in the animal facility at Yarmouk University, specifically in an animal room where standard laboratory conditions were maintained. These conditions included a temperature range of 20-22°C, relative humidity between 60-80%, and a lighting cycle of 12 hours of light/ 12 hours of darkness. The study protocol was approved by the Department Research Committee at Yarmouk University.

2.3.1. The median lethal dose (LD_{50})

To measure the median lethal dose (LD_{50}) of *A. orientalis* leaf extracts, 60 mice were utilized. The animals underwent an overnight fast lasting approximately 14 hours. They were then distributed among 10 groups, with each group consisting of 6 mice per group. Both extracts were administered intraperitoneally (IP) for 24 hours at varying doses, with each group receiving a daily single injection of one of the following doses: 5000, 4000, 2000, 1000, and 500 mg/kg. Mortality cases or indications of toxicity were monitored in the tested mice and the lowest dose concentration that killed 50% of mice in the tested groups indicated the value of LD_{50}.

2.3.1. Micronucleus (MN) formation, and total antioxidant capacity

In vivo animal study was conducted using 66 *Balb/c* male mice to assess the genotoxic potential of *A. orientalis* leaf extracts. After one week of environmental acclimation, mice were divided into a total of 11 groups (n=6 mice/group). Four groups were assigned to the aqueous extraction method, and four groups were assigned to the ethanolic extraction method. Each group received a daily dose of one of the following concentrations: 4000, 2000, 1000, and 500 mg/kg of plant extract given via the IP route of injection. Additionally, two groups served as solvent negative controls (distilled water and 20% ethanol), and one group for the positive control (mitomycin C, 14 mg/kg, Shabbar *et al*., 2012). Both treatment and control experiments were run simultaneously and terminated after 28 days. At study termination, and after 30 hours of injection, the mice were euthanized by cervical dislocation. The ethylenediaminetetraacetic acid (EDTA) as an anticoagulant was used to collect peripheral blood samples. In addition, the liver of mice was collected in pre-sterilized tubes.

2.4. Micronucleus assay

2.4.1. Blood smears preparation

Clean pre-washed glass slides were used to prepare the blood smears. From each mouse, three slides were created, and these blood films were allowed to air-dry. Subsequently, they were fixed in absolute methanol for a duration of 3 minutes, following the methods described by Heddle in [26] and Schmid in [27]

2.4.2. Staining and MN evaluation

All the prepared slides were double-stained with Mayer's hematoxylin (BioGnost, Zagreb) for 10 minutes and then 10% Giemsa (GCC, UK) for 20 minutes [28]. The slides were thoroughly rinsed in tap water, followed by a 10-minute differentiation step in Sorensen's buffer (pH 6.8). Afterward, they were left to air-dry. Observations were conducted using

a BioBlue light microscope (Euromex, Netherlands) equipped with an oil immersion lens (100X). For each treatment, 2000 normochromic erythrocytes from each animal were screened for MN formation.

2.5. TAC assay

To measure the total antioxidant capacity of *A. orientalis* leaf extracts, MyBioSource CheKine ™ Total TAC assay kit (Cat. number MBS9718973, lot. number ATTJL2301, USA) was used. To conduct this test, liver samples were collected in pre-sterilized tubes. Subsequently, 0.1 gram of each liver sample was homogenized with 1 ml of assay buffer, and the resulting mixture was centrifuged at 12,000 x, at 4°C for 10 minutes. The supernatant was then transferred to a new tube and kept on ice for further detection. For sample detection, 10 µl of each sample was combined with 150 µl substrate diluent, 15 µl substrate, and 15 µl reaction buffer. The sample mixtures were then incubated in a 96-well plate at room temperature for 5 minutes, and the optical density (O. D) was measured at 593 nm. The Total Antioxidant Capacity (TAC) for each sample (expressed in U/g) was determined using the following formula:

$$TAC(U/g) = \frac{0.6 \times (O.D_{\ sample} - O.D_{\ blank})}{(O.D_{standard} - O.D_{blank})} \times \frac{1}{W}$$

Where: *O.D* is the optical density, *W* is the weight of the sample in grams.

2.6. Statistical analysis

The data underwent analysis through a one-way analysis of variance (*ANOVA*) using SPSS, version 26, USA. The data were presented as mean values ± standard error of the mean. Following the ANOVA, Tukey's multiple comparisons tests were conducted as a post-hoc analysis. Data were deemed statistically significant when the *p* value was less than 0.05.

3. RESULTS

3.1. The median lethal dose LD_{50}

After a 24-hour period following a single injection, there were no indications of toxicity or mortality within the dosage range of 500-2000 mg/kg for both leaf extract methods. However, at higher doses of 4000 and 5000 mg/kg, the mortality rate resulting from intraperitoneal administration increased progressively with the rising dose, as detailed in Table (1). Nonetheless, the mortality percentage at the dose of 4000 mg/kg was equal for both aqueous and ethanolic extract methods (Fig.1). Conversely, at the highest tested dose (5000 mg/kg), the number of mouse fatalities was greater in the aqueous extraction group compared to the ethanolic group. The median lethal dose LD_{50} of *A. orientalis* extract was detected in the experimental group of mice that were administered a dose of 4000 mg/kg for both types of extracts, as indicated in Figure (1).

Table 1. Results of the tested doses of A. orientalis extract used to ascertain the LD_{50} following intraperitoneal injection in Balb/c male mice.

Extract	Group	N. of Mice	Doses (mg/kg)	Death number	Mortality %
Aqueous	A	6	500	0	0
	B	6	1000	0	0
	C	6	2000	0	0
	D	6	4000	3	50
	E	6	5000	5	83.3
Ethanolic	A	6	500	0	0
	B	6	1000	0	0
	C	6	2000	0	0
	D	6	4000	3	50
	E	6	5000	4	66.7

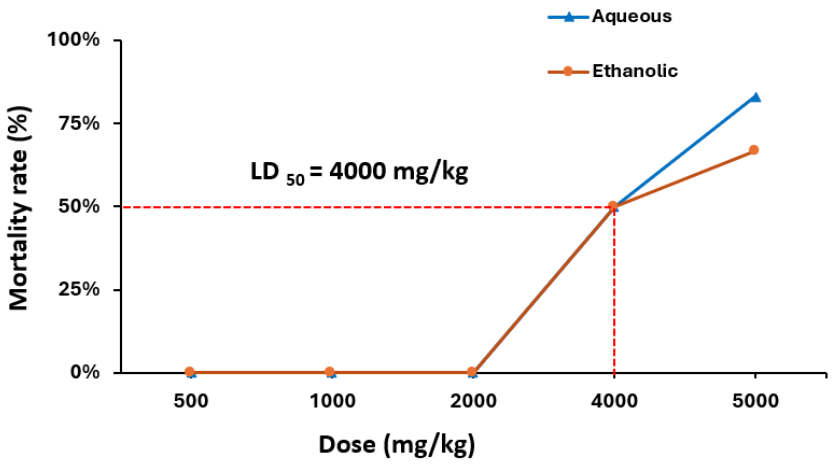


Figure 1. Dose-response (mortality rate %) curve. The red dashed line represents the LD $_{50}$.

3.2 MN assay

The percentage of micronuclei was determined after scoring approximately 2000 cells. As demonstrated in Table (2), a noticeable increase in the percentage of micronucleus (MN) formation was observed as the extract doses increased for both types of extracts. However, it's worth mentioning that this percentage was slightly higher in the aqueous extract (decoction). The highest observed percentages of MN were 0.0679% and 0.052% at the dose of 4000 mg/kg for the aqueous and ethanolic extracts, respectively. The percentage of micronucleus (MN) formation at a dose of 4000 mg/kg for the aqueous extract represents a substantial increase when compared to the control groups (distilled water and 20% ethanol), with percentages of 0.001% and 0.007%, respectively. For instance, a noticeable disparity in the incidence of MN formation was evident between the mice group that received 2000 mg/kg and the negative control group treated with the aqueous extract (Figure 1). This discrepancy in the means of MN formation subsequent to the administration of *A. orientalis* extracts was found to be statistically significant ($p < 0.05$) when compared to both the negative and positive control groups (Table 2).

Table 2. the micronucleus (MN) formation observed in *Balb/c* mice at various concentrations of *A. orientalis* extract.

	Aqueous extract		**Ethanolic extract**	
Treatment	Mean ± S.D $^{\Sigma}$	Freq. (%) ± S.D	Mean ± S.D $^{\Sigma}$	Freq. (%) ± S.D
N. control	15.75 ± 2.5	0.001 ± 0.001	14.8 ± 3.962	0.007 ± 0.002
P. control (MMC)	79.5 ± 5.259	0.04 ± 0.003	79.5 ± 5.259	0.04 ± 0.003
500 mg/kg	53.8 ± 5.263$^{*,\#}$	0.027 ± 0.003	44.2 ± 3.193$^{*,\#}$	0.022 ± 0.002
1000 mg/kg	83 ± 4.743$^{*,\#}$	0.042 ± 0.002	71.6 ± 2.873$^{*,\#}$	0.036
2000 mg/kg	93.66 ± 7.359$^{*,\#}$	0.046 ± 0.004	86 ± 4.516$^{*,\#}$	0.043 ± 0.002
4000 mg/ kg	135.83 ± 3.656$^{*,\#}$	0.0679 ± 0.002	103 ± 3.741$^{*,\#}$	0.052 ± 0.002

"Σ" denotes the total count of cells with micronuclei (MN) in normochromatic erythrocytes, "#" is used to denote statistical significance ($p < 0.05$) when compared with the positive group, while "*" is used to denote statistical significance ($p < 0.05$) when compared with the negative group. MMC is the abbreviation of mitomycine C.

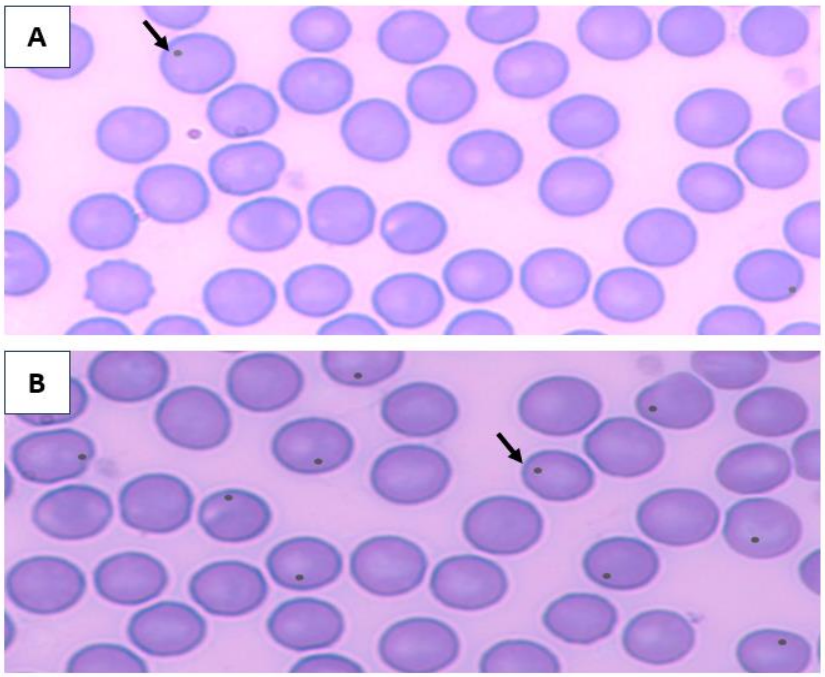


Figure 1. Representative photomicrographs of mouse blood smear stained with 10% Giemsa, (A) negative control group (B) 2000 mg/kg group of aqueous extract. The arrow indicates MN formation.

3.3 TAC assay

Table (3) presents the outcomes of Total Antioxidant Capacity (TAC) in the liver cells of mice following the administration of varying doses of aqueous and ethanolic *A. orientalis* extracts. For both types of extracts, the mean ± standard deviation (SD) values of the experimental groups exhibited an increase with rising doses. It is worth noting that the TAC, an indicator of the total antioxidant capacity of the extracts, was higher in the ethanolic extract as compared to the aqueous extract. Furthermore, both extracts exhibited lower antioxidant capacity across all concentrations compared to the positive and negative control groups. In the case of the aqueous extract, the results indicated statistically significant differences ($p < 0.05$) in the means of TAC for all doses when compared to both the negative and positive control groups. On the other hand, for the ethanolic plant extract, the group that received the lowest dose (4000 mg/kg) exhibited a TAC value that was in proximity to the positive control value ($p > 0.05$).

Table 3. The levels of TAC in *Balb/c* mice at different concentrations of *A. orientalis* extract.

	Aqueous extract	**Ethanolic extract**
Treatment	Mean ± S.D $^{\Sigma}$	Mean ± S.D $^{\Sigma}$
N. control	158.350 ± 0.572	169.9 ± 3.140
P. control (MMC)	156.38 ± 0.571	156.325 ± 0.665
500 mg/kg	84.150 ± 0.641*, #	130.967 ± 3.149*, #
1000 mg/kg	89.472 ± 12.366*, #	135 ± 0.969*, #
2000 mg/kg	92.917 ± 8330*, #	146.40 ± 0.550*, #
4000 mg/ kg	116.90 ± 8.894*, #	155.34 ± 0.5550

"Σ" denotes the total amount of TAC (U/ g), "#" is used to denote statistical significance ($p < 0.05$) when compared with the positive group, while "*" is used to denote statistical significance ($p < 0.05$) when compared with the negative group. MMC is the abbreviation of mitomycine C.

4. DISCUSSION

The utilization of medicinal plants as the primary means of medical treatment has been a historical and enduring practice in developing nations [29]. Plant-based natural products remain an abundant source of novel phytochemicals and nutraceuticals [30]. It's important to note that some of these phytochemicals found in medicinal plant extracts may be associated with toxicity [31]. Hence, it is crucial and advisable to evaluate the toxicity of medicinal plant extracts during the preclinical assessment phase [11]. The current study demonstrates that the aqueous and ethanolic extracts obtained from *Ajuga orientalis* leaves exhibit toxicity when administered through the intraperitoneal (IP) route in mice, with toxic effects observed at doses of 2000 and 4000 mg/kg. Notably, mortality was only observed in mice when relatively high doses of both extracts were intraperitoneally injected, resulting in an LD_{50} value of 4000 mg/kg for these extracts. In a closely related and commonly used medicinal plant, *Ajuga iva*, a previous study by El Hilaly [32] found that the LD50 for acute intraperitoneal administration in mice and rats was 3600 mg/kg. Given that closely related plants tend to share similar or identical chemical constituents, this suggests that phytotoxicity is often consistent at the genus level [33].

One of the primary and recommended tests for evaluating product safety is the in vivo assessment of micronucleus formation (MN) frequencies [34]. Consequently, the utilization of MN as an indicator of chromosomal damage has become a widespread method for assessing genotoxicity and conducting human biomonitoring research [35]. In our research, we observed a dose-dependent increase in the percentage of MN formation in bone marrow cells of mice with escalating concentrations of the extracts. This rise in MN-containing erythrocytes among treated mice was statistically significant for both the aqueous and ethanolic extracts when compared to the control groups (both negative and positive). Such a dose-related increase in MN formation is considered an indicative sign of genotoxicity activity [34]. Where Phytochemicals present in plants are among the genotoxic agents known to induce MN formation [36], which can subsequently lead to DNA or chromosomal damage [37]. Several previous studies have explored the genotoxic effects of various plant species. These investigations have found that extracts from plants such as *Crataegus oxyacantha, Aristolochia debilis, Asristolochia heterophylla, Astagalus membranaceus, Bupleurum talcatum, Canrthamus tinctorius, Cinnamomum mairie, and Cuscuta chinensis* can increase the percentage of MN formation in peripheral cells in a dose-dependent manner [9,38]. Consequently, our results suggest that both extracts derived from *A. orientalis* leaves have a significant impact on the induction of MN formation, indicating high genotoxicity, particularly at higher doses. Nevertheless, it's important to note that in human use, exposure to crude extracts of medicinal plants typically occurs orally, often in the form of decoctions, rather than through injection. Therefore, the dose, extraction method, and route of administration for herbal medicines are crucial factors that should be thoroughly investigated for their potential toxicity and safety implications [2].

Despite the widespread use of *A. orientalis* in traditional medicine, the extracts derived from its leaves exhibited low antioxidant capacity. In general, both extracts showed significantly lower antioxidant activity compared to the control groups. This outcome aligns with similar findings reported in studies on various folk medicinal *Ajuga* species that used different assays, such as *A. orientalis* [1], *A. bracteosa* [39], *A. parviflora* [40], and *A. reptans* [41]. On the other hand, the choice of extraction method, specifically the solvent used, influenced the total antioxidant capacity (TAC). In the case of *A. orientalis* leaves, the ethanolic extract exhibited higher TAC compared to the aqueous extract. This trend has also been observed in the aforementioned studies on *Ajuga* species. Generally, the addition of 20%–60% ethanol or methanol to distilled water can significantly enhance the antioxidant

capacity during plant extraction [42]. This enhancement is attributed to their ability to extract higher levels of phenolic and flavonoid compounds while reducing the consumption rate of endogenous antioxidants, thus acting as antioxidants [43]. It's important to emphasize the significance of natural antioxidants, as they are regarded as a preventive approach against chronic diseases due to their capacity to reduce oxidative stress and enhance immune function [44]. Furthermore, natural antioxidants can contribute to improving healthcare systems by reducing the dependency on costly and frequently inefficacious treatments. In the specific case of our study species, *A. orientalis*, the low antioxidant capacity of its leaf extracts suggests that it may not be a promising natural source of antioxidants and may have limited potential as a medicinal plant in this regard.

5. CONCLUSIONS

In summary, the findings derived from this study indicate that *A. orientalis* leaf extracts display genotoxic potential while exhibiting a relatively modest antioxidant capacity. Both the aqueous and ethanolic extracts of *A. orientalis* leaves induced a statistically significant increase in micronuclei (MN) formation in normochromic erythrocytes among treated mice, following a dose-dependent pattern. Therefore, it underscores the critical importance of considering dosage and extraction methods in the evaluation of potential toxicity and safety associated with herbal medicines. This research underscores the imperative need for further investigations to thoroughly evaluate the safety and toxicity of *A. orientalis*, particularly focusing on its active chemical constituents that may induce potential genotoxic effects. This is especially pertinent given its continued utilization in traditional medicinal practices.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Oran S.A., Althaher A.R. and Shhab M.A. Al Chemical Composition , in Vitro Assessment of Antioxidant Properties and Cytotoxicity Activity of Ethanolic and Aqueous Extracts of Ajuga Orientalis L . (Lamiaceae). *J. Pharm. Pharmacogn. Res.* 2022; *10*, 486–495.
2. Mensah M.L.K., Komlaga G., Forkuo A.D., Firempong C., Anning K., Komlaga G., Dickson R.A., Forkuo D., Firempong C., Anning K., et al. Toxicity and Safety Implications of Herbal Medicines Used in Africa Toxicity and Safety Implications of Herbal Medicines Used in Africa. In *Herbal Medicine*; Builders, P.F., Ed.; *IntechOpen*, 2019.
3. Kahaliw W., Hellman B. and Engidawork E. Genotoxicity Study of Ethiopian Medicinal Plant Extracts on HepG2 Cells. *BMC Complement. Altern. Med.* 2018; *18*: 1–9. doi: 10.1186/s12906-017-2056-x.
4. V K. *Toxicological Survey of African Medicinal Plants*; V, K., Ed.; 1st ed.; London: *Elsevier Inc*. 2014.
5. Ferreira-machado S.C., Rodrigues M.P., Nunes A.P.M., Bezerra R.J.A.C., Caldeira-de-araujo A. Genotoxic Potentiality of Aqueous Extract Prepared from Chrysobalanus Icaco L . Leaves. *Toxicol. Lett.* 2004. *151*: 481–487, doi: 10.1016/j.toxlet.2004.03.014.
6. Bardoloi A. and Soren A.D. Genotoxicity Induced by Medicinal Plants. *Bull. Natl. Res. Cent.* 2022. doi: 10.1186/s42269-022-00803-2.
7. Melo-Reis P., Bezerra L., Vale M., Canhête R., Chen-Chen L. Assessment of the Mutagenic and Antimutagenic Activity of Synadenium Umbellatum Pax Latex by Micronucleus Test in Mice. *Brazilian J. Biol.* 2011; *71*: 169–174. doi: 10.1590/s1519-69842011000100024.

8. Verschaeve L. NPC Natural Product Communications. *Nat. Prod. Commun.* 2015; *10*: 1489–1493. doi: 10.1177/1934578X1501000843.
9. Yonekubo B.T., Miranda H. D.e., Alves C., Marques D.S., Perazzo F.F., César P., Rosa P., Gaivão O.N.D.M., Maistro E.L., Marques S., et al. The Genotoxic Effects of Fruit Extract of Crataegus Oxyacantha (Hawthorn) in Mice. *J. Toxicol. Environ. Heal. Part A.* 2018; *81*: 974–982. doi: 10.1080/15287394.2018.1503982.
10. Shin S., Yi J.M., Kim N.S., Chan-Sung Park Kim S.H., Ok-Sun Bang Aqueous Extract of Forsythia Viridissima Fruits: Acute Oral Toxicity and Genotoxicity Studies. *J. Ethnopharmacol.* 2020; *249*: 112381. doi: 10.1016/j.jep.2019.112381.
11. Harutyunyan K., Balayan K., Tadevosyan G., Hayrapetyan M., Musayelyan R., Grigoryan R., Khondkaryan L., Sarkisyan N. and Babayan N. Genotoxic Potential of Selected Medicinal Plant Extracts in Human Whole Blood Cultures. *J. HerbMed Pharmacol.* 2019; *8*: 160–162, doi: 10.15171/jhp.2019.25.
12. Adwan G. In Vitro Assessment of Antibacterial Activity and Potential Genotoxic Effect of Fruit Extracts of Capparis Spinosa L . Plant. *Jordan J. Pharm. Sci.* 2023; *16*: 322–329.
13. Oran S.A. The Status of Medicinal Plants in Jordan. *J. Agric. Sci. Technol. A.* 2014; *4*, 461–467.
14. Abu-Irmaileh B.E. and Afifi F.U. Herbal Medicine in Jordan with Special Emphasis on Commonly Used Herbs. *J. Ethnopharmacol.* 2003; *89*: 193–197. doi: 10.1016/S0378-8741(03)00283-6.
15. El-alali A., Zoubi A. A.l., Gharaibeh M., Tawaha K. and Alali F.Q. Phytochemical and Biological Investigation of Nitraria Retusa Asch. *Jordan J. Pharm. Sci.* 2012; *5*: 155–163.
16. Uritu C.M., Mihai C.T., Stanciu G.D., Dodi G., Alexa-Stratulat T., Luca A., Leon-Constantin M.M., Stefanescu R., Bild V., Melnic S., et al. Medicinal Plants of the Family Lamiaceae in Pain Therapy: A Review. *Pain Res. Manag.* 2018; *2018*. doi: 10.1155/2018/7801543.
17. Abu-Odeh A., Fino L., Al-Absi G., Alnatour D., Al-Darraji M., Shehadeh M. and Suaifan G. Medicinal Plants of Jordan: Scoping Review. *Heliyon.* 2023; *9*: e17081. doi: 10.1016/j.heliyon.2023.e17081.
18. Brahmi F., Khaled-khodja N., Bezeghouche R., Bouharis S., Elsebai M.F., Madani K. and Boulekbache-makhlouf L. Ethnobotanical Study of the Most Lamiaceae Used as Medicinal and Culinary Plants by the Population of Bejaia Province , Algeria. *Jordan J. Pharm. Sci.* 2023; *16*: 268–281.
19. Taha-Salaime L., Davidovich-Rikanati R., Sadeh A., Abu-Nassar J., Marzouk-Kheredin S., Yahyaa Y., Ibdah M., Ghanim M., Lewinsohn E., Inbar M., et al. Phytoecdysteroid and Clerodane Content in Three Wild Ajuga Species in Israel. *ACS Omega.* 2019; *4*: 2369–2376. doi: 10.1021/acsomega.8b03029.
20. Göger F., Köse Y.B., Göger G. and Demirci F. Phytochemical Characterization of Phenolics by LC-MS/MS and Biological Evaluation of Ajuga Orientalis from Turkey. *Bangladesh J. Pharmacol.* 2015; *10*: 639–644.
doi: 10.3329/bjp.v10i3.23500.
21. Luan F., Han K., Li M., Zhang T., Liu D., Yu L. and Lv H. Ethnomedicinal Uses, Phytochemistry, Pharmacology, and Toxicology of Species from the Genus Ajuga L.: A Systematic Review. *Am. J. Chin. Med.* 2019; *47*: 959–1003. doi: 10.1142/S0192415X19500502.
22. Sajjadi S.E. and Ghannadi A. Volatile Oil Composition of the Aerial Parts of Ajuga Orientalis L . from Iran §. *Zeitschrift für Naturforsch.* 2004; *59*: 166–168.
23. Gautam R., Jachak S.M. and Saklani A. Anti-Inflammatory Effect of Ajuga Bracteosa Wall Ex Benth. Mediated through Cyclooxygenase (COX) Inhibition. *J. Ethnopharmacol.* 2011; *133*: 928–930.
doi: 10.1016/J.JEP.2010.11.003.

24. Zengin G., Ceylan R., Katani J., Aktumsek A., Mati S., Boroja T., Mihailovi V., Seebaluck-sandoram R., Mollica A. and Fawzi M. Exploring the Therapeutic Potential and Phenolic Composition of Two Turkish Ethnomedicinal Plants – Ajuga Orientalis L . and Arnebia Densi Fl Ora (Nordm .). *Ind. Crop. Prod.* 2018; 116: 240–248. doi: 10.1016/j.indcrop.2018.02.054.
25. Shabbar I.M.S., Maslat A., Shabbar I.M.S., Genotoxicity A.M., Rich L.A., Fruit C. and Using J. Genotoxicity of Ecballium Elaterium (L) A Rich Cucurbitaceae Fruit Juice Using Micronucleus Assay and DNA Single Strand Break Techniques. *Internet J. Heal.* 2012; 6: 1–12. doi: 10.5580/1b75.
26. Heddle J.A., Hite M., Kirkhart B., Mavournin K., MacGregor J.T., Newell G.W. and Salamone M.F. The Induction of Micronuclei as a Measure of Genotoxicity. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res. Genet. Toxicol.* 1983; *123*: 61–118. doi: 10.1016/0165-1110(83)90047-7.
27. Schmid W. The Micronucleus Test. *Mutat. Res. Mutagen. Relat. Subj.* 1975; 31: 9–15. doi: 10.1016/0165-1161(75)90058-8.
28. Khalil A.M., Salman W.K. and Al-Qaoud K.M. Evaluation of Genotoxic Potential of 2-(Bromoacetamido) Phenylboronic Acid on Balb/C Mice Peripheral Blood Cells Using in Vivo Micronucleus Assay. *Jordan J. Pharm. Sci.* 2017; 10: 103–112. doi: 10.12816/0040698.
29. Petrovska B.B. Historical Review of Medicinal Plants' Usage. *Pharmacogn. Rev.* 2012; 6: 1–5. doi: 10.4103/0973-7847.95849.
30. Ahmad R., Ahmad N., Naqvi A.A., Shehzad A. and Al-Ghamdi M.S. Role of Traditional Islamic and Arabic Plants in Cancer Therapy. *J. Tradit. Complement. Med.* 2017; 7: 195–204. doi: 10.1016/j.jtcme.2016.05.002.
31. Oloya B., Namukobe J., Ssengooba W., Afayoa M. and Byamukama R. Phytochemical Screening, Antimycobacterial Activity and Acute Toxicity of Crude Extracts of Selected Medicinal Plant Species Used Locally in the Treatment of Tuberculosis in Uganda. *Trop. Med. Health.* 2022; *50*. doi: 10.1186/s41182-022-00406-7.
32. El Hilaly J., Israili Z.H. and Lyoussi B. Acute and Chronic Toxicological Studies of Ajuga Iva in Experimental Animals. *J. Ethnopharmacol.* 2004; *91*: 43–50. doi: 10.1016/j.jep.2003.11.009.
33. Hao D., cheng and Xiao P. gen Pharmaceutical Resource Discovery from Traditional Medicinal Plants: Pharmacophylogeny and Pharmacophylogenomics. *Chinese Herb. Med.* 2020; *12*: 104–117. doi: 10.1016/j.chmed.2020.03.002.
34. Rohit Sahu, Goli Divakar, K.D. IN VIVO RODENT MICRONUCLEUS ASSAY OF GMELINA ARBOREA ROXB (GAMBHARI) EXTRACT. *J. Adv. Pharm. Technol. Res.* 2010; *1*: 22–29.
35. Kwasniewska J. and Bara A.W. Plant Cytogenetics in the Micronuclei Investigation—The Past, Current Status, and Perspectives. *Int. J. Mol. Sci.* 2022; *23*. doi: 10.3390/ijms23031306.
36. Luzhna L., Kathiria P. and Kovalchuk O. Micronuclei in Genotoxicity Assessment : From Genetics to Epigenetics and Beyond. *Front. Genet.* 2013; *4*: 1–17. doi: 10.3389/fgene.2013.00131.
37. Phillips D. and Arlt V. Genotoxicity: Damage to DNA and Its Consequencese. In *Molecular, Clinical and Environmental Toxicology.* 2009; 87–110.
38. Zhou J., Ouedraogo M., Qu F. and Duez P. Potential Genotoxicity of Traditional Chinese Medicinal Plants and Phytochemicals: An Overview. *Phyther. Res.* 2013; *27*: 1745–1755.doi: 10.1002/ptr.4942.

39. Kayani W.K., Dilshad E., Ahmed T., Ismail H. and Mirza B. Evaluation of Ajuga Bracteosa for Antioxidant, Anti-Inflammatory, Analgesic, Antidepressant and Anticoagulant Activities. *BMC Complement. Altern. Med.* 2016; *16*: 1–13. doi: 10.1186/s12906-016-1363-y.
40. Suryavanshi A., Kumar S., Kain D. and Arya A. Chemical Composition, Antioxidant and Enzyme Inhibitory Properties of Ajuga Parviflora Benth. *Biocatal. Agric. Biotechnol.* 2021; *37*: 102191. doi: 10.1016/j.bcab.2021.102191.
41. Esposito T., Sansone F., Auriemma G., Franceschelli S., Pecoraro M., Picerno P., Aquino R.P. and Mencherini T. Study on Ajuga Reptans Extract: A Natural Antioxidant in Microencapsulated Powder Form as an Active Ingredient for Nutraceutical or Pharmaceutical Purposes. *Pharmaceutics.* 2020; *12*: 1–23. doi: 10.3390/pharmaceutics12070671.
42. Venkatesan T., Choi Y.W. and Kim Y.K. Effect of an Extraction Solvent on the Antioxidant Quality of Pinus Densiflora Needle Extract. *J. Pharm. Anal.* 2019; *9*: 193–200. doi: 10.1016/j.jpha.2019.03.005.
43. Bonnefont-Rousselot D., Rouscilles A., Bizard C., Delattre J., Jore D. and Gardès-Albert M. Antioxidant Effect of Ethanol toward in Vitro Peroxidation of Human Low-Density Lipoproteins Initiated by Oxygen Free Radicals. *Radiat. Res.* 2001; *155*: 279–287. doi: 10.1667/0033-7587(2001)155[0279: AEOETI]2.0.CO;2.
44. Khlebnikov A.I., Schepetkin I.A., Domina N.G., Kirpotina L.N. and Quinn M.T. Improved Quantitative Structure-Activity Relationship Models to Predict Antioxidant Activity of Flavonoids in Chemical, Enzymatic, and Cellular Systems. *Bioorganic Med. Chem.* 2007; *15*: 1749–1770. doi: 10.1016/j.bmc.2006.11.037.

تقييم السمية الجينية والقدرة المضاداة للأكسدة في مستخلصات أوراق عشبة الدم الشرقية في الفئران *Ajuga orientalis* L. (Lamiaceae)

محمد الغرايبة[1*]، احمد مسلط[2]، نور اليقين كناكري[2]، يزن جرار[3]

[1] قسم الانتاج النباتي، كلية الزراعة، جامعة العلوم والتكنولوجيا الاردنية، الأردن.

[2] قسم العلوم الحياتية، كلية العلوم، جامعة اليرموك، الأردن.

[3] قسم العلوم الطبية الاساسية، كلية الطب، جامعة البلقاء التطبيقية، الأردن.

ملخص

يعد نبات عشبة الدم الشرقية من النباتات العطرية المستوطنة لمنطقة شرق البحر الابيض المتوسط والتي تستخدم بشكل شائع في الطب الشعبي في الاردن والدول المجاورة. على الرغم من استعمالها الواسع، إلا أنه لا توجد دراسات سمية على مستخلصات اوراقها. تهدف هذه الدراسة لتقييم سمية هذا النبات في مسخلصات الاوراق عن طريق الايثانول والماء لتقييم باستخدام اختبار تكوين الأنوية الصغيرة في كريات الدم الحمراء الصبغية السوية في الحمض النووي الريبوزي منقوص الأكسجين للفئران (DNA) وايضا لتقييم إجمالي نشاط القدرة المضادة للأكسدة. لتحديد الجرعة القاتلة الوسيطة (LD_{50}) لمستخلصات أوراق نبات الدم الشرقي، تم اعطاء تراكيز متفاوته ل 66 من ذكور فأر *Balb/c*. تم حقن الفئران يوميا بداخل البطن بالتراكيز التالية 4000 و2000 و1000 و500 ملغ/كغ وتم استخدام 3 مجموعات كضوابط ولمدة 28 يوماً. تم فحص تكون الأنوية الصغيرة في كريات الدم الحمراء العادية، وتم تقييم عينات الكبد لقياس القدرة الكلية المضادة للأكسدة.كان تركيزالجرعة القاتلة الوسيطة لكلا المستخلصين 4000 ملغ/كغ، مع زيادة معنوية في تكوين الأنوية الصغيرة في كريات الدم الحمراء العادية كلما زاد تركيز الجرعة. اظهرت النتائج انخفاض في قيم إجمالي نشاط القدرة المضادة للأكسدة في كلا المستخلصين مقارنة بالضوابط. تشير النتائج إلى وجود سمية جينية لمستخلصات أوراق نبات الدم الشرقي في الفئران ، مما يحث على الحذر في استهلاكها من قبل البشر. يجب إجراء المزيد من الأبحاث لتقييم سلامتها وسميتها بشكل شامل، خاصةً مع استخدامها التقليدي الواسع في الطب الشعبي.

الكلمات الدالة: الأنوية الصغيرة، نبات الدم الشرقي، نبات طبي، إجمالي نشاط القدرة المضادة للأكسدة.

---

[*] ***المؤلف المراسل: محمد الغرايبة***

mfagharaibeh@just.edu.jo

تاريخ استلام البحث 2024/03/07 وتاريخ قبوله للنشر 2024/06/14.

Jordan Journal of Pharmaceutical Sciences, Volume 17, No. 4, 2024

Evaluating the Nutritional and Chemical Composition of *Treculia Africana* and *Vigna Subterranea* L. Seeds Collected from Kogi State, Nigeria

Eneogwe Okechukwu Godfrey*[*1], Okpala Onwudiegwu Ejike[1], Anthony William Ojoniko[1], Ibrahim Izihyi Esther[2], Obuye Faith[1], Atumeyi Anthony Ugbedeojo[1] and Abdullahi Idris Bilkisu[1]

[1]Department of Chemistry, Faculty of Science, Federal University Lokoja, Nigeria.
[2]Department of Industrial Chemistry, Faculty of Science, Federal University Lokoja, Nigeria.

ABSTRACT

This study aims to investigate the nutritional composition of two underutilized leguminous crops, namely *Treculia africana* and *Vigna subterranea* seeds, collected from Kogi state, Nigeria. The study analysed their proximate composition, mineral content, and amino acid profile using standard analytical methods. Additionally, the chemical composition of the sample was determined using gas chromatography-mass spectroscopy. The results showed that there were significant differences ($P \leq 0.05$) in the legume samples. However, *V. subterranea* seeds had the least moisture content (12.90±0.81 %) as well as the highest crude fat content, crude fiber content, crude protein and ash content at 15.70±0.41 %, 5.06±0.16 %, 27.86±0.25 % and 3.23±0.50 %, respectively. The elemental analysis in mg/100g indicated that the samples contained appreciable levels of essential minerals. *T. africana* had the highest magnesium, phosphorus, sodium, potassium and iron concentrations of 190.03±1.70 mg/100g, 315.95±1.60 mg/100g, 32.61±1.82 mg/100g, 1941.53±2.61 mg/100g and 39.50±1.73 mg/100g, respectively, while calcium (58.46±1.63) was most abundant in *V. subterranea* The samples were also rich in amino acids, which are the building blocks of protein. However, *V. subterranea* was the richest in amino acid content, as it had 33.07±2.22 g/100g and 46.01±4.24 g/100g, for essential and non-essential amino acids, respectively. The GC-MS characterization of the chemical composition of the samples showed that myristic acid (48.1) was the most abundant in *T. africana*, while ethyl palmitate (31.17) was the most abundant in *V. subterranea.* Overall, the results suggest that these legume samples are rich sources of both nutritional and pharmaceutical properties beneficial for human consumption.

Keywords: Amino acid profile, chemical composition, mineral concentration, proximate composition, *Treculia africana*, *Vigna subterranean*.

1. INTRODUCTION

Africa still faces a major issue with food and nutritional insecurity, with two hundred and thirty-two million individuals suffering from micronutrient deficiencies and around two hundred and thirty-nine million suffering from protein-calorie malnutrition [1]. Meat, dairy and seafood are costly for low-income households in rural Africa, even though they can help prevent protein-calorie malnutrition. Therefore, protein-rich plant foods have been the primary choice for many underprivileged African populations [1]. Legumes are a great source of inexpensive plant proteins and minerals compared to animal products and are the second most significant food crop in the tropics, after cereals [2]. Hence, natural legumes offer a valuable and affordable alternative source of protein for low-income populations in many developing nations, particularly in

[*]***Corresponding author: Eneogwe Okechukwu Godfrey***
godfrey.eneogwe@fulokoja.edu.ng
Received: 16/03/2024 Accepted: 14/06/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2480

© 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

Asia and Africa where grains are a staple food [2].

The seeds of two native leguminous crops, *T. africana* (African breadfruit) and *V. subterranea* (Bambara nut) have great promise as sources of nutrients and useful chemicals. These crops are still underutilized and poorly researched despite their high nutritional content and capacity to adapt to local environmental conditions [3]. Thus, assessing their chemical and nutritional composition is crucial to encouraging their use as food resources and improving food security in areas where they are grown, like Kogi State, Nigeria. Through the use of a multidisciplinary approach to evaluate the nutritional and chemical composition of samples primarily from Kogi State, Nigeria, this study provides new insights into the potential benefits of *T. africana* and *V. subterranea* seeds for food security, nutrition and regional economic development.

Bambara nut is native to Africa. It is highly suited to the sub-Saharan African agroclimatic conditions, which are often harsh [3]. Bambara nuts have a desirable nutritional composition comprising minimal fat, a decent amino acid profile, a high protein and carbohydrate content [4]. The high nutritional value of Bambara nut, especially its essential amino acid content, including methionine, lysine and leucine as well as its adaptation to harsh environmental conditions makes it a valuable crop for food and nutrition security, especially in sub-Saharan Africa, where nutrient deficiencies affect the majority of the population [4]. Furthermore, it has been discovered that Bambara nuts have significant antioxidant qualities, which suggests they may have positive health effects [5].

The African breadfruit is one of the four genera and members of the family Moraceae. It is home to numerous tropical nations, including the West Indies, Ghana, Sierra Leone, Nigeria and Jamaica. *T. africana* is a delicacy among the Igbo people of southeast Nigeria. The plant is well known in southeast Nigeria as "ukwa." The biological value of the seeds is higher than even that of soybeans, and they have a remarkable polyvalent dietetic value [6]. It is one of the most prized commercial plants and a highly regarded medicinal plant used in most traditional herbal medicine treatments. It's considered high in fiber, vitamins, minerals, fat, carbs and high-quality protein. Significant concentrations of phytochemicals, including flavonoids, polyphenols, anthraquinones, saponins, and cardiac glycosides, are also present in it [6]. In traditional medicine, the plant's crude extracts alone or in conjunction with other herbs, have been used to cure various illnesses.

In a world where dietary habits are changing and people are becoming more aware of how food choices affect their health, the nutritional analysis of African breadfruit and Bambara nuts becomes germane. Therefore, by investigating the nutritional and chemical composition of *T. africana* and *V. subterranea* collected from Kogi State, Nigeria, this research harmonises traditional knowledge with contemporary nutritional science, thus closing the divide between traditional knowledge and modern understanding. This study investigates the proximate composition, mineral concentration, amino acid profile and chemical composition of *T. africana* and *V. subterranea* collected from Kogi State, Nigeria.

2. MATERIALS AND METHODS

2.1 Materials

Every chemical compound, reagent and solvent used in the experiment was acquired from Sigma-Aldrich (St. Louis, MO, U.S.A.). These included petroleum ether, sulfuric acid, boric acid, hydrolysate, hydrochloric acid, nitric acid and perchloric acid. All of the reagents were of analytical reagent grade.

2.2 Instruments

A Scitek Atomic Absorption Spectrometer, Model SP-AA3618, was used to analyse the samples for mineral content. This equipment has an air/acetylene burner, eight lamp supports, and a double beam. It also features a 350 mm focal length Czerny-Turner type monochromator. This spectrometer has a wavelength range of 185-900 nm, a wavelength repeatability of $\leq$ 0.05 nm and a wavelength

precision of 0.15 nm. The exterior dimensions are 830 x 650 x 60 (W x D x H), and the spectral bandwidth variation is ±0.02 nm. The device makes use of deuterium background correction to guarantee accuracy. The photometer's optics are also shielded from corrosive fumes and dust.

A Technicon Sequential Multi-Sample (TSM) amino acid analyzer (Technicon Instrument Corporation, New York) was used to measure the amino acids, and the analytical techniques used followed the manufacturer's recommendations. The Technicon Sequential Multi-Sample (TSM) is fitted with two short columns, 35 × 0.6 cm, with adjustable bottom fittings. The resins (C-4 chromobeads) have an average particle size of 12 µm. The resin bed is 21 × 0.6 cm and operates at a temperature of 61ºC. It has a flow rate of 0.80ml/min and an operating pressure of 200-250 psi (1379-1724 kN/m^2).

The GC-MS Agilent Technologies, Model-7890A, the Gallenkamp oven (Model: 800-NE1V.32E-00), the Lenton furnaces, England, the water bath (Memmert model WTB24), the soxhlet extractor, and the Kjeldahl flask were additional instruments utilised.

2.3 Sample collection

Randomly selected matured accessions of *T. africana* and *V. subterranea* were collected from nearby farms in Lokoja, Kogi State, communities of Adankolo and Zangondaji, from September 4th to 8th 2023. The samples were cleared of soil particles and transported to the laboratory for analysis at a tropical ambient temperature.

2.4 Sample preparation

The Bambara nut (*V. subterranea*) and African breadfruit (*T. africana*) seeds were sorted, and foreign objects and rotten grains were eliminated. The seeds were hand-separated from their shells and allowed to air dry for three days. The dried African breadfruit and Bambara nut grains were then crushed into a powder using a pestle and mortar and sieved into fine flour using a 250 µm sieve.

2.5 Determination of Proximate Composition

Standard analytical techniques as described by the Association of Official Analytical Chemists (AOAC) [7] were applied to ascertain the moisture content, ash content, crude protein, crude fiber and percentage of fat. The carbohydrate content was determined by difference. Using the formula: $\%\ Carbohydrate\ content = 100 - (\%\ moisture + \%\ ash + \%\ crude\ protein + \%\ crude\ fiber + \%\ crude\ fat)$

2.6 Determination of Mineral Concentration

Samples were digested according to the AOAC [7] protocol for mineral analysis. After the sample was pulverised and weighed, 1.00 g was added to a 250 mL beaker. The beaker was filled with a strong acid mixture of 15.00 mL HNO_3 and 5.00 mL perchloric acid. The mixture was thoroughly mixed to guarantee appropriate mixing and then heated on a hot plate until a clear digest emerged. After letting the digest cool, it was quantitatively filtered into a 100 mL volumetric flask. To aspirate the filtrate into the AAS machine (Scitek Atomic Absorption Spectrometer, Model SP-AA3618) for trace metal analysis, it had to be produced up to the 100 mL threshold.

2.7 Determination of Amino Acid Profiles

To evaluate the sample, a Soxhlet extractor was used to extract 3.00 g of the sample for six hours at 40–60°C in petroleum ether, following the method described by Cooper *et al.* [8]. After extraction, a glass ampoule was filled with 30.00 mg of defatted samples and 7.00 mL of 6.00 mol/dm^3 hydrochloric acid. Nitrogen was released from the ampoule after oxygen was supplied to avoid the oxidation of some amino acids during hydrolysis. The ampoule was then sealed with a Bunsen flame and placed in a preheated oven at 105°C for 22 hours. After cooling, the tip of the ampoule was broken and its contents were filtered. The filtrate was then evaporated to dry at 40°C while vacuuming in a rotating evaporator. The residue was later dissolved in 5.00 mL of pH 2.0 acetate buffer and stored for 24 hours in a plastic bottle in the deep freezer. Finally, 5-10 microliters of the hydrolysate were added to the Technicon Sequential Multi-Sample (TSM) amino acid analyzer (Technicon Instrument Corporation, New York). The analysis took 76 minutes to complete once the sample

was placed into the analyzer's cartridge.

2.8 Chemical composition of the samples using Gas chromatography-mass spectrometry (GC-MS) analysis

T. africana and *V. subterranea* were subjected to chemical composition analysis using the methodology outlined by Karki *et al.* [9]. The GC-MS analysis was conducted using Agilent Technologies Model-7890A gas chromatograph and 5975C mass spectrometer. For the gas chromatography analysis, an HP-5MS capillary column was used, which was 30 meters long, 0.320 mm in diameter internally, and had a 0.25 µm film thickness. Helium was used as the carrier gas with a pressure of 1.5 PSI, a constant flow rate of 1.4123 mL/min and an average velocity of 43.311 cm/sec. The temperature was programmed to start at 80 degrees Celsius for two minutes, then increase to 240 degrees Celsius at 10 degrees Celsius per minute. The injected samples had a volume of 1 µL, a split ratio of 50:1 and a split flow rate of 70.615 mL/min. The constituents were identified by comparing the published mass spectrum database (NIST 11. L) and literature data with the auto-integrated whole chromatogram.

2.9 Statistical Analysis

To assess the degree of significance between various samples, the obtained findings were statistically analysed using mean standard deviation and analysis of variance (ANOVA), as explained by Duncan's multiple range test. Significance was determined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Proximate composition of *V. subterranea* and *T. Africana* samples

Table 1 shows the results of the proximate composition analysis of *V. subterranea* and *T. africana. V. subterranea* and *T. africana's* moisture content are 12.90±0.81 % and 38.90±0.76 % respectively. There is a significant difference ($p \leq 0.05$) between the moisture content of both samples, with *V. subterranea* having lower moisture content. This result is consistent with James *et al.* [2] study, which found that the moisture content of some lesser-known native legumes in Nigeria ranged from 9.30±0.00 % to 12.75±0.00 %. Compared to *T. africana*, *V. subterranea* is more resistant to deterioration and has a longer shelf life [2].

The ash content of *T. africana* and *V. subterranean* are 0.50±0.05% and 3.23±0.50% respectively. There was a significant difference ($p \leq 0.05$) between the two samples, with *V. subterranea* having the highest ash content. This finding is approximately similar to the study conducted by Musah *et al.* [10], which reported a 3.40±0.09% ash content for Bambara groundnut in Lapai, Nigeria. According to Godfrey *et al.* [11], this suggests that *V. subterranea* has a higher mineral content than the other sample analysed.

The crude fat content for *T. africana* and *V. subterranea* are 1.93±0.12 % and 15.70±0.41 % respectively. A significant difference ($p \leq 0.05$) was observed between the samples, with *V. subterranea* exhibiting the highest fat content. Nonetheless, the outcome is comparable to the study conducted by Abejide *et al.* [12], which revealed that the crude fat content of several Bambara nut claims varied, ranging from 9.88±0.17 % to 15.83±0.01 %. The fact that dietary fat supplies most of the energy required by man suggests that *V. subterranea* is a better source of calories than *T. africana* [13].

The crude fiber content for *T. africana* and *V. subterranea* are 4.76±0.25 % and 5.06±0.16 % respectively. There was a significant difference ($p \leq 0.05$) across the samples, with the maximum fiber content found in *V. subterranea* The outcome, however, is approximal to the study by James *et al.* [2], which found that the crude fiber content of a few lesser-known native legumes from Nigeria ranged from 2.33±0.01 % to 6.11±0.01 %. This suggests that compared to the other sample examined, *V. subterranea* is more suited to reduce the risk of obesity, heart disease, diabetes and softens stool [14].

The crude protein content for *T. africana* and *V. subterranea* are 16.53±0.42 % and 27.86±0.25 % respectively. There was a significant difference ($p \leq 0.05$)

across the samples, with the maximum fibre content found in *V. subterranea.* However, the outcome is approximated to that of Mohammed and Mhya [15] analysis, which found that Bambara groundnut varieties cultivated in north-eastern Nigeria had yields ranging from 19.5 % to 21.1 %. This indicates that *V. subterranea* is the better sample among the two examined for tissue growth and healing [16].

The carbohydrate content for *V. subterranea* and *T. africana* are 35.24±1.56 % and 37.38±1.12 % respectively. There was a significant difference ($p \leq 0.05$) between the samples, with *T. africana* exhibiting a higher carbohydrate content. However, the outcome is comparable to that of the Frances and Johnson [17] study, which had 38.39 % for *T. africana* seeds. This suggests that, compared to the other samples examined, *T. africana* seeds are considered to be a superior source of energy for all species [17].

Table 1. Proximate composition of *T. africana* and *V. subterranea* samples

Sample	Moisture content (%)	Ash content (%)	Crude Fat content (%)	Crude Fibre content (%)	Crude Protein content (%)	Carbohydrate (%)
Treculia Africana	38.90±0.76	0.50±0.05	1.93±0.12	4.76±0.25	16.53±0.42	37.38±1.12
Vigna subterranea L.	12.90±0.81	3.23±0.50	15.70±0.41	5.06±0.16	27.86±0.25	35.24±1.56

Note: Values are means ± standard deviation of triplicate analysis

3.2 Mineral concentration of the *T. africana* and *V. subterranea* samples

The results of the mineral concentration of *T. africana* and *V. subterranea* are shown in Table 2. The calcium content for *T. africana* and *V. subterranea* are 42.90±1.60 mg/100g and 58.46±1.63 mg/100g respectively. There was a significant difference ($p \leq 0.05$) among the samples, with the maximum calcium concentration found in *V. subterranea.* The result, however, is approximately similar to the 37 mg/100 g to 64 mg/100 g calcium concentration of *V. subterranea* found from various landraces in Namibia reported by Amarteifio *et al.* [18]. Calcium is necessary for blood coagulation, tooth and bone growth, muscle contraction and neurological function [19]. It is recommended that adults take 1000 mg of calcium per day, while children should take 500–800 mg [20].

The magnesium content for *V. subterranea* and *T. africana* are 124.00±1.84 mg/100g and 190.03±1.70 mg/100g respectively. There was a significant difference ($p \leq 0.05$) between the samples, with *T. africana* exhibiting the highest magnesium concentration. However, the outcome is near that of Ojimelukwe and Ugwuona [21] analysis, which found that raw *T. africana* seeds contained 186.00 mg/100g. Magnesium is linked to immunologic dysfunction, poor spermatogenesis, alopecia, dermatitis, muscle degeneration, development retardation and fetal abnormalities [22]. The recommended daily intake of magnesium is 80–320 mg [20].

The phosphorous content for *V. subterranea* and *T. africana* is 78.64±1.80 mg/100g and 315.95±1.60 mg/100g respectively. There was a significant difference ($p \leq 0.05$) across the samples, with *T. africana* exhibiting the highest iron concentration. Nonetheless, the outcome is comparable to the 395.66±0.01 mg/100g phosphorus concentration of *T. africana* from Nigeria reported by James *et al.* [2]. Particularly in young children and nursing moms, phosphorus helps to strengthen bones and teeth [23]. The recommended daily intake of magnesium for both adults and children is 800 mg [20].

The sodium content for *V. subterranea* and *T. africana* are 21.39±1.40 mg/100g and 32.61±1.40 mg/100g respectively. There was a significant difference ($p \leq 0.05$)

between the samples, with *T. africana* exhibiting the highest sodium concentration. Nonetheless, the result is more than that of Frances and Johnson [17] research, which stated that *T. africana* seeds contained 15.50 mg/100g. Sodium, a macronutrient, plays a vital role in the body's metabolic activities by exciting and transmitting nerve impulses during action [24]. According to WHO [20], the recommended daily salt intake for adult males is 10 mg, while for females, it is less than 15 mg.

The potassium content for *V. subterranea* and *T. africana* are 622.60±1.61 mg/100g and 1941.53±1.80 mg/100g, respectively. There was a significant difference ($p \leq 0.05$) between the samples, with *T. africana* showing the highest potassium concentration. However, the result is higher than that of Ojimelukwe and Ugwuona [21] analysis, which found that raw *T. africana* seeds contained 186.00 mg/100g. Potassium is essential for maintaining the body's water balance, neurotransmission, immunological response, and signalling [25]. The WHO [20] recommends a daily intake of 2000 mg of potassium for adults and 1000 mg for children.

The iron content for *V. subterranea* and *T. africana* are 10.75±1.32 mg/100g and 39.50±1.50 mg/100g, respectively. There was a significant difference ($p \leq 0.05$) across the samples, with *T. africana* showing the highest iron concentration. Nonetheless, the result is approximately similar to the 4.06±0.01 mg/100g to 17.93±0.01 mg/100g reported by James *et al*.[2] for the iron content of a few lesser-known Nigerian native legumes. Iron is present in considerable amounts in haemoglobin, a protein found in red blood cells that carries oxygen from the lungs to every body part [26]. It is advised that adults take 17.0–18.9 mg of iron per day, while children should take 13.7–15.1 mg [20].

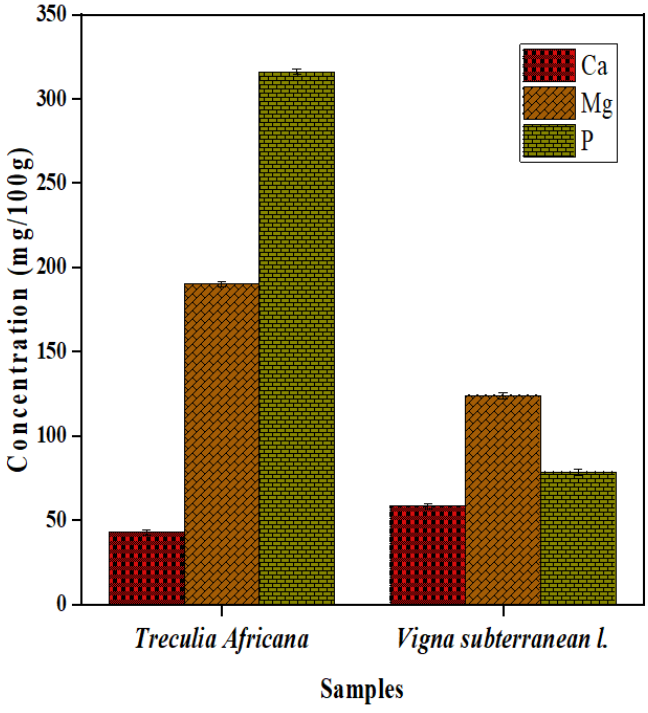


Fig 1. The mineral concentration (calcium, magnesium and phosphorous) of *T. africana* and *V. subterranean* seeds.

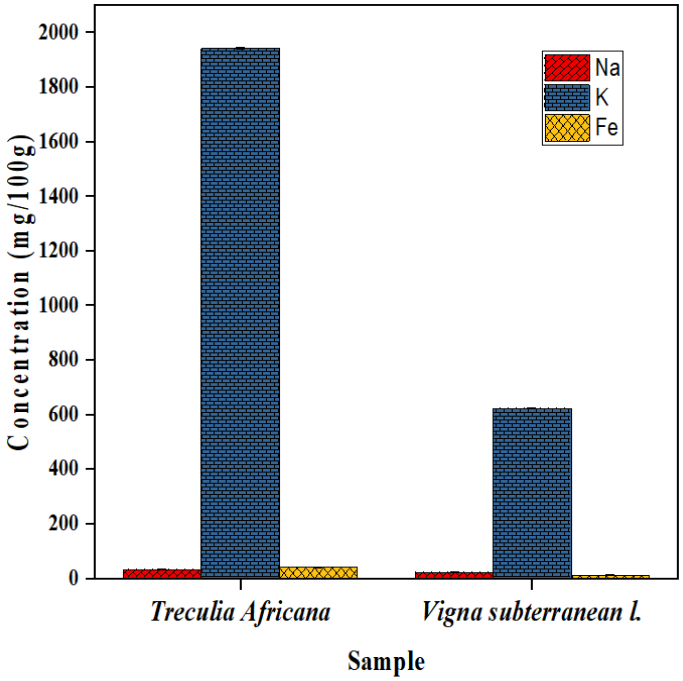


Fig 2. The mineral concentration (sodium, potassium and iron) of *T. africana* and *V. subterranea* seeds.

3.3 Amino acid profile of the *T. africana* and *V. subterranean* samples

The results of the amino acid profiles of *T. africana* and *V. subterranea* are shown in Table 2. Amino acids are the building blocks of proteins, which also have vital bodily functions. According to the research, legumes are a good source of essential and non-essential amino acids. Among the necessary amino acids are Lysine, phenylalanine, valine, threonine, isoleucine, methionine, histidine and leucine. The non-essential amino acids are alanine, arginine, aspartic acid, serine, tyrosine, proline, cysteine and glutamic acid. Nonetheless, results demonstrated that in *T. africana* and *V. subterranea* seed samples, the non-essential amino acids exceeded the essential amino acids. The total non-essential amino acid values for *T. africana* and *V. subterranea* seeds were 30.29±3.55 g/100g and 46.01±4.24 g/100g, respectively, representing 57.85 % and 58.18 %. The total essential amino acid levels for *T. Africana* and *V. subterranea* Seeds were 22.07±1.63 g/100g and 33.07±2.22 g/100g, respectively, representing 42.15 % and 41.82 %. This outcome is comparable to studies published by Musah *et al*. [10].

V. subterranean had the highest non-essential amino acid and essential amino acid content at 46.01±4.24 g/100g and 33.07±2.22 g/100g respectively. *T. Africana* had the least non-essential amino acid and essential amino acid content at 30.29±3.55 g/100g and 22.07±1.63 g/100g respectively. Furthermore, the percentage ratio of the essential amino acid (EAA) to the total amino acid (TAA) in the samples is 42.15 % and 41.82 %. These values are well above the 39%, which is considered adequate for ideal protein food for infants, 26% for children and 11% for adults.

Glutamic acid appeared to be the most abundant amino acid in *T. africana* and *V. subterranea* Seeds at 10.57±0.31 g/100g and 15.41±0.58 g/100g, respectively. By producing glutathione, a potent antioxidant essential for bolstering the immune system and shielding cells from oxidative stress, glutamic acid contributes to the immune system's

support. In addition, it functions as a neurotransmitter and is necessary for detoxification, protein synthesis and preserving the body's acid-base equilibrium [27].

Tryptophan a precursor to the neurotransmitter serotonin, which controls mood, hunger and sleep, was found in meagre amounts in all the samples, with *T. africana* being the least abundant at 0.01±0.00 g/100g [28]. At 0.69±0.10 g/100 g, cystine was the least abundant amino acid in *V. subterranea*, yet it is important for protein synthesis and redox balance maintenance [29]. Additionally, it helps with wound healing, immune system function, healthy skin and hair, wound healing and the metabolism of fatty acids. It is beneficial in detoxifying hazardous compounds such as heavy metals and environmental pollutants [30].

The minimum amino acid intake of 1.5 g/kg/day is reported to be necessary for preventing negative nitrogen balance, while 2.5 g/kg/day is not advisable [31].

Table 2. Amino acid profile of *T. africana* and *V. subterranea* seeds

Amino acids	*T. Africana* (g/100g)	*V. subterranea* (g/100g)
Leucine	4.87±0.15	7.25±0.19
Lysine	3.79±0.22	4.13±0.25
Isoleucine	1.70±0.18	1.82±0.07
Phenylalanine	1.72±0.11	5.24±0.32
Tryptophan	0.01±0.00	0.91±0.08
Valine	1.85±0.31	5.96±0.10
Methionine	1.50±0.24	0.92±0.05
Histidine	1.92±0.13	3.30±0.11
Threonine	4.71±0.14	3.54±0.12
*Proline	5.52±0.10	6.48±0.27
*Arginine	3.87±0.15	4.69±0.11
*Tyrosine	0.62±0.11	2.09±0.15
*Cystine	0.85±0.14	0.69±0.10
*Alanine	0.58±0.33	4.89±0.13
*Glutamic acid	10.57±0.31	15.41±0.58
*Glycine	0.92±0.18	2.75±0.28
*Serine	0.85±0.13	4.05±0.33
*Aspartic acid	6.51±0.25	4.96±0.24
TEAA	22.07±1.63 (42.15%)	33.07±2.22 (41.82%)
TNEAA	30.29±3.55 (57.85%)	46.01±4.24 (58.18%)

Note: Values are means ± standard deviation of triplicate analysis, TEAA - Total essential amino acid, TNEAA - Total non-essential amino acid, *Non-essential amino acid

3.4 The chemical composition of *T. africana* and *V. subterranea* sample

The results of the chemical composition analysis of *T. africana* and *V. subterranea* seeds using GC-MS are presented in Tables 3 and 5 while, the chemical composition summary is shown in Tables 4 and 6. In *T. africana* seeds, 24 compounds were identified, accounting for 99.43% of the total composition, while in *V. subterranea* seeds, 18 compounds were identified, accounting for 95.90% of the total composition. The most abundant compounds in *T. africana* seeds were Myristic acid (48.01%), linoleic acid (28.27%), and palmitic acid (13.94%), while in *V. subterranea* seeds, the most abundant compounds were ethyl palmitate (31.17%) and ethyl stearate (13.08%). *T. africana* and *V. subterranea* seeds contained different classes of compounds, with *T. africana* seeds dominated by fatty acid

and fatty acid esters (92.21%), alkanes and alkenes (4.18%), and monoterpenes (1.35%), while *V. subterranea* seeds were dominated by fatty acid and fatty acid esters (60.55%), alkanes (27.20%), ketones (5.64%), and esters (2.51%). Comparing the composition of *T. africana* and *V. subterranea* seeds, myristic acid and ethyl palmitate were found to be the major components in both samples, with a higher content of 48.01% and 31.17%, respectively. The comparison of the composition pattern of *T. africana* and *V. subterranea* seeds showed notable qualitative and quantitative differences. The *T. africana* seeds have nutraceutical uses in improving cholesterol levels, heart health, and digestion, reducing inflammation, improving skin health, boosting the immune system, and reducing the risk of certain types of cancer, such as breast cancer, which may be attributed to the presence of myristic acid, the major chemical constituent in *T. africana* seeds [32]. Myristic acid is a saturated fatty acid found in many foods, including dairy products, nutmeg, and coconut oil [33]. Ethyl palmitate, the most abundant chemical constituent found in *V. subterranea* seeds is often used in cosmetic and skincare products for their emollient properties. It helps soften and smooth the skin, enhancing the texture and feel of skincare formulations [34]. Ethyl palmitate is sometimes used as a fragrance carrier in cosmetic products [35]. Contrary to popular opinion, ethyl palmitate has pharmaceutical importance as it has anti-inflammatory properties [36]. Apart from its applications in pharmaceuticals, ethyl palmitate is used in the wine industry to enhance flavours, as an additive in tobacco for cigarette making and in the food industry for processing nut products [37].

Table 3. Chemical composition of African breadfruit (*T. africana*)

S/N	RT (min)	Compounds	Composition (%)	Formula	Class of compound
1	3.751	Limonene	1.02	$C_{10}H_{16}$	Monoterpenes
2	8.052	d-α-Pinene	0.33	$C_{10}H_{16}$	Monoterpenes
3	8.561	trans-4-Tetradecene	0.32	$C_{14}H_{28}$	Alkenes
4	8.660	Undecane	0.22	$C_{11}H_{24}$	Alkane
5	9.282	1-Cyclohexyloctane	0.15	$C_{14}H_{28}$	Cycloalkane
6	10.118	2,4-Di-tert-butylphenol	0.43	$C_{14}H_{22}O$	Phenol
7	11.036	Cyclohexadecane	1.07	$C_{16}H_{32}$	Cycloalkane
8	11.114	Hexadecane	0.22	$C_{16}H_{34}$	Alkane
9	11.768	1-Decylcyclohexane	0.18	$C_{16}H_{32}$	Cycloalkane
10	13.013	Myristic acid	0.42	$C_{14}H_{28}O_2$	Fatty acid
11	13.267	1-Octadecene	1.44	$C_{18}H_{36}$	Alkene
12	13.329	10-Methylnonadecane	0.31	$C_{20}H_{42}$	Alkanes
13	14.009	Hexane, 1,6-dicyclohexyl	0.27	$C_{18}H_{34}$	Cycloalkane
14	14.056	Pentadecylic acid	0.30	$C_{15}H_{30}O_2$	Fatty acid
15	14.108	Octyl ketone	0.37	$C_{17}H_{34}O$	Ketone
16	14.626	Methyl palmitate	0.31	$C_{17}H_{34}O_2$	Fatty acid methyl ester
17	15.311	Palmitic acid	13.94	$C_{16}H_{32}O_2$	Fatty acid
18	15.524	Myristic acid	48.01	$C_{14}H_{28}O_2$	Fatty acid
19	16.308	Methyl linoleate	0.36	$C_{19}H_{34}O_2$	Fatty acid methyl ester
20	16.359	trans-13-Octadecenoic acid, methyl ester	0.45	$C_{19}H_{36}O_2$	Fatty acid methyl ester
21	16.583	Hexadecylic acid	0.15	$C_{16}H_{32}O_2$	Fatty acid
22	17.174	Linoleic acid	28.27	$C_{18}H_{32}O_2$	Fatty acid
23	20.106	4-Methylindole	0.31	C_9H_9N	Indole
24	22.747	Naphthalene, 1,2,3,4-tetrahydro-1- octyl-	0.58	$C_{18}H_{28}$	PAHs

Key: RT = Retention time

Table 4. Summary of chemical composition of African breadfruit (*T. africana*)

S/N	Class of compounds	% Composition
1	Monoterpenes	1.35
2	Alkanes and alkenes	4.18
3	Phenols	0.43
4	Fatty acid and fatty acid esters	92.21
5	Ketones	0.37
6	Indole	0.31
7	Polycyclic aromatic hydrocarbons (PAHs)	0.58
8	Total	99.43

Table 5. Chemical composition of Bambara nut (*V. subterranea*)

S/N	RT (min)	Compounds	Composition (%)	Formula	Class of compounds
1	12.307	5-Methyloctadecane	2.38	$C_{19}H_{40}$	Alkane
2	13.277	Ethyl undecanoate	2.51	$C_{13}H_{26}O_2$	Esters
3	13.324	Octadecane	1.77	$C_{18}H_{38}$	Alkane
4	13.822	6,10,14-Trimethyl-2-pentadecanone	5.64	$C_{18}H_{36}O$	Ketone
5	14.351	Hexadecane	2.30	$C_{16}H_{34}$	Alkane
6	14.626	Methyl palmitate	3.23	$C_{17}H_{34}O_2$	Fatty acid methyl ester
7	15.109	Palmitic acid	4.41	$C_{16}H_{32}O_2$	Fatty acid
8	15.348	Ethyl palmitate	31.17	$C_{18}H_{36}O_2$	Fatty acid ethyl ester
11	16.282	Heneicosane	4.47	$C_{21}H_{44}$	Alkane
12	16.915	Palmitoleic acid	4.28	$C_{16}H_{30}O_2$	Fatty acid
13	16.982	Ethyl elaidate	4.38	$C_{20}H_{38}O_2$	Fatty acid ethyl ester
14	17.169	Ethyl stearate	13.08	$C_{20}H_{40}O_2$	Fatty acid ethyl ester
15	17.195	Hexadecane	3.50	$C_{16}H_{34}$	Alkane
16	18.041	Heptadecane	4.10	$C_{17}H_{36}$	Alkane
17	18.980	9-Methylnonadecane	5.30	$C_{20}H_{42}$	Alkane
18	20.126	Tricosane	3.38	$C_{23}H_{48}$	Alkane

Key: RT = Retention time

Table 6. Summary of chemical composition of Bambara nut (*V. subterranea*) seeds

S/N	Class of compounds	% Composition
1	Alkanes	27.20
2	Esters	2.51
3	Ketones	5.64
4	Fatty acid and fatty acid esters	60.55
5	Total	95.90

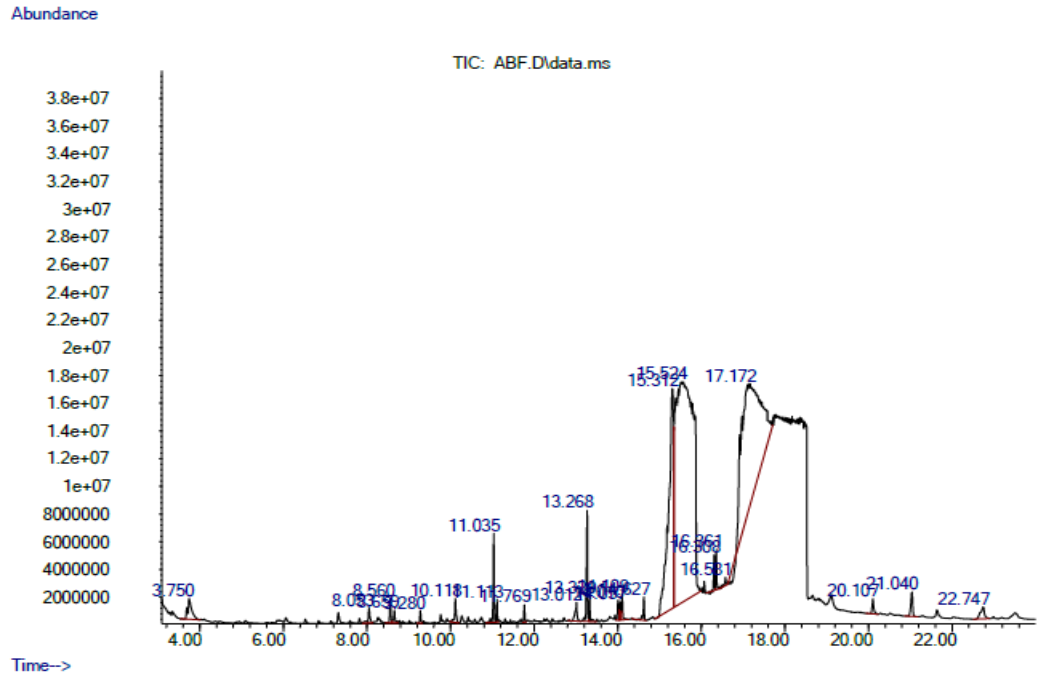


Fig 3. GC-MS spectra showing the chemical composition of African breadfruit (*T. Africana*) seeds

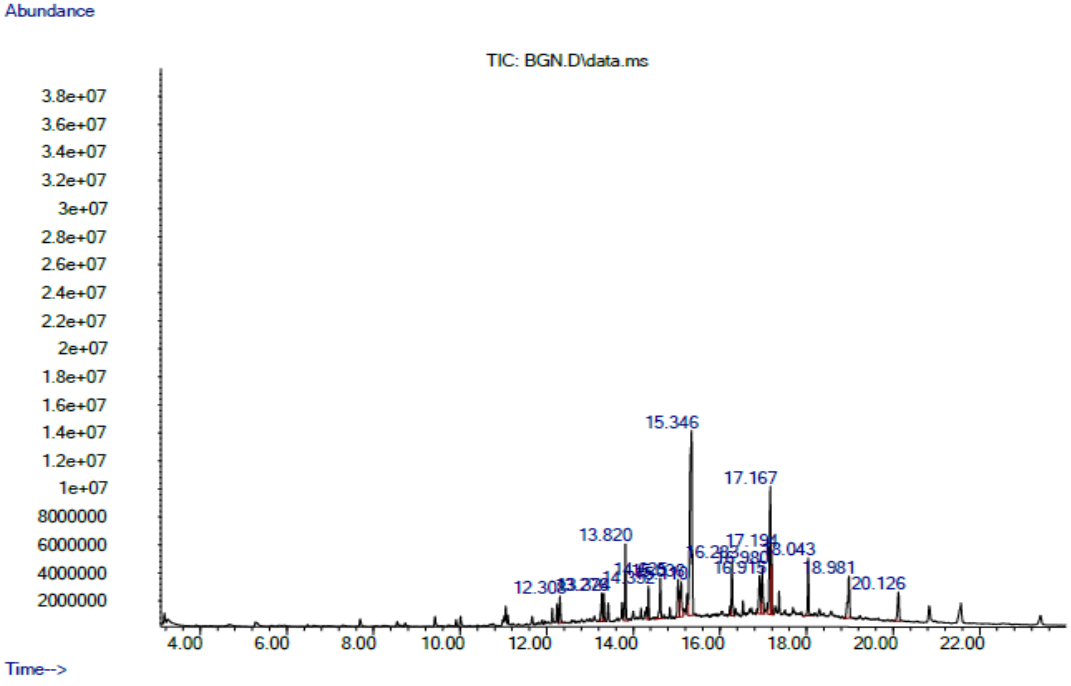


Fig 4. GC-MS spectra showing the chemical composition of Bambara nut (*V. subterranea*) seeds.

4. CONCLUSION

This study aimed to investigate two types of legumes, *T. africana* and *V. subterranea*, for their nutritional and chemical composition. The proximate composition analysis was used to determine the distribution of macronutrients in the legumes, shedding light on their potential as dietary sources. The results showed that *V. subterranea* seeds had the highest levels of ash, crude fat, crude fiber and crude protein content, while *T. africana* seeds had the highest carbohydrate content. Regarding mineral content, *T. africana* had the highest concentration of magnesium, phosphorous, sodium, potassium and iron, while calcium was most abundant in *V. subterranea* These disparities in mineral content provide valuable insight into the diverse nutritional benefits offered by these legumes as well as the extent to which they play crucial roles in various physiological functions within the human body. The amino acid profile of both legumes indicated they are rich in protein, with *V. subterranea* being the richest in amino acid content. Moreover, both legumes contained significant amounts of macro and micro-nutrients essential to human nutrition. The findings of the gas chromatography-mass spectrometry (GC-MS) analysis shed light on the chemical diversity of the samples analysed, underscoring their potential as valuable sources of bioactive compounds with implications for food, pharmaceutical and nutraceutical industries. The result showed that 24 compounds were identified in *T. africana* seeds with Myristic acid being the most abundant compound, while 18 compounds were identified in *V. subterranea* seeds, with ethyl palmitate being the most abundant compound. The findings of this research could provide valuable insights into the potential uses of these legumes in functional foods and nutraceuticals.

ACKNOWLEDGEMENTS

The authors acknowledge the contributions of all research technologists of the Department of Chemistry and Industrial Chemistry, Federal University Lokoja, Kogi State, Nigeria during the laboratory and statistical analysis.

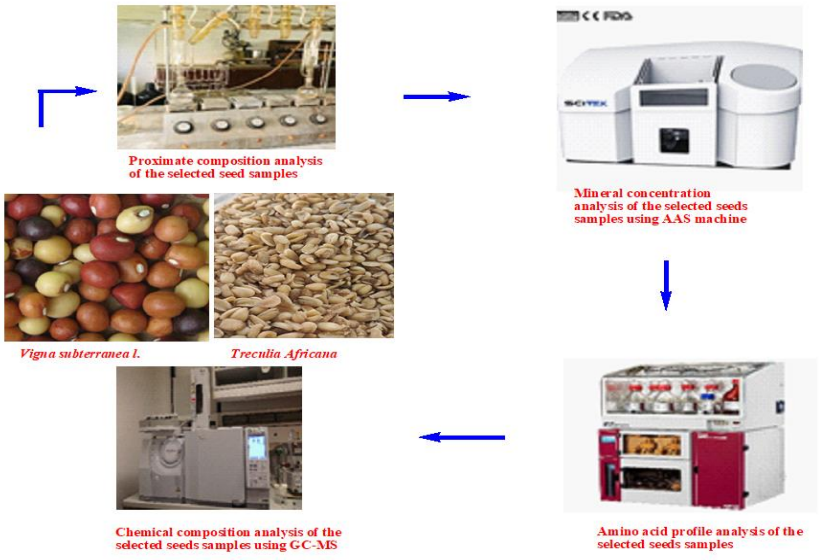
COMPETING INTEREST DISCLAIMER

The authors declare that they have no conflict of interest in this study.

FUNDING

This research did not receive any funding

Graphical abstract:



REFERENCES

1. Dakora F. D. and Belane A. K. Evaluation of protein and micronutrient levels in edible cowpea (*Vigna Unguiculata L. Walp*.) leaves and seeds. *Frontiers in Sustainable Food Systems*. 2019; 3: 1-10. https://doi.org/10.3389/fsufs.2019.00070
2. James S., Ugochukwu T., Onwuka G. I., Ndife J. and Ata M. Chemical and nutritional composition of some selected lesser-known legumes indigenous to Nigeria. *Heliyon*. 2020; 6(11):e05497. https://doi.org/10.1016/j.heliyon.2020.e05497
3. Oyeyinka S. A., Ajisafe A. O., Akintayo O. A., Iyiola O. A. Oyeyinka A. T., Olatunde S. J., Badmos A. H. A., Fasogbon B. M. and Patel N. Effect of microwave treatment on cooking time, colour, sensory and nutritional properties of Bambara groundnut (Vigna subterranea). *Croatian Journal of Food Technology, Biotechnology and Nutrition*. 2020; 15 (1–2):11–16.
4. Mashau M. E., Mukwevho T. A., Eugenia S. and Siwela M. The influence of Bambara groundnut (Vigna subterranean) flour on the nutritional, physical and antioxidant properties of steamed bread nutritional, physical and antioxidant properties of steamed bread. *CyTA - Journal of Food*. 2022; 20(1):259–270. https://doi.org/10.1080/19476337.2022.2130435
5. Oyeyinka A. T., Pillay K. And Siwela M. In vitro digestibility, amino acid profile and antioxidant activity of cooked Bambara groundnut grain. *Food Bioscience*. 2019; 31:100428. https://doi.org/10.1016/j.fbio.2019. 100428
6. Osukoya O. Nwoye-Ossy M. Olayide I. Ojo O. Adewale O. and Kuku A. Antioxidant activities of peptide hydrolysates obtained from the seeds of Treculia africana Decne (African breadfruit). *Preparative Biochemistry & Biotechnology*. 2020; 50(5):504-510.
7. AOAC. Association of Official Analytical Chemists. *Official method of analysis (19th edition).* 2006; Gaithersburg: AOAC Press, USA.
8. Cooper C. (Ed.). *Amino acid analysis protocols*. 2008; (159). Springer Science & Business Media.
9. Karki D., Pandey B., Jha P., Acharya A., Khanal D., Raut B. and Panthi S. Senna alata: Phytochemistry, Antioxidant, Thrombolytic, Anti-inflammatory, Cytotoxicity, Antibacterial activity, and GC-MS analysis. *Jordan Journal of Pharmaceutical Sciences*. 2023; 17(3):549–566. https://doi.org/10.35516/jjps.v17i3.2406
10. Musah M., Azeh Y., Mathew J. T., Nwakife N. C., Mohammed A. I. and Saidu F. Nutritional evaluation of Bambara groundnut (Vigna subterranea (L.) Verdc) from Lapai, Nigeria. *African Journal of Agriculture and Food Science*. 2021; 4(4):32-39.
11. Godfrey E. O. Faith O. and Esther I. I. Comparative Assessment of the Proximate Composition, Functional Properties and Amino Acid Profile of Dioscorea bulbifera, Dioscorea alata and Dioscorea rotundata Found in Minna, Niger State. *Biology, Medicine, & Natural Product Chemistry*. 2023; 12(1):177–185.
12. Abejide D. R. Samson O. Ehoniyotan O. I. Olubiyo G. T. Onemayin D. Y. Proximate composition of some Nigerian Bambara groundnut (Vigna subterranea (L.) Verdc.) accessions. *GSC Biological and Pharmaceutical Sciences*. 2023; 24(1):339-345.
13. Godfrey E. O. Esther I. I. and Faith O. Proximate Composition, Levels of Some Essential Mineral Elements and Anti-Nutritional Components of Some Yam Species Found in Minna, Niger State. *Biology, Medicine, & Natural Product Chemistry*. 2022; 12(1):9–16. https://doi.org/10.14421/biomedich.2023.121.9-16
14. Nkwocha Chinelo C., Nworah Florence N., Okagu Innocent U. and Nwagwe Onyinyechi R. Proximate and Phytochemical Analysis of Monodora myristica (African Nutmeg) from Nsukka, Enugu State, Nigeria. *Journal of Food and Nutrition Research*. 2018; 6(9):597- 601.

15. Mohammed A. and Mhya H. D. Evaluation of phytochemical constituents, proximate contents and glycemic index of bambara groundnut (Vigna subterranea L. Verdc) varieties grown in Northeastern Nigeria. *African Journal of Biochemistry Research*. 2021; 15(2): 22-27.
16. Yahya I. H., Abd Hamid H. and Iwansyah A. C. (2024). Evaluation of Proximate Composition, Multielement, and Bioactive Phenolics Contents of Different Coix Seed Varieties using Multivariate Analysis Techniques. *Jordan Journal of Pharmaceutical Sciences*. *17*(3):492-505.
17. Frances E. C. and Johnson O. O. Assessment of Proximate, Vitamins and Minerals of African Breadfruit Seed (Treculia africana). *Journal of Global Ecology and Environment*. 2022; 16(2):28-36.
18. Amarteifio J. O. Tibe V. and Njogu R. M. The mineral composition of bambara groundnut (Vigna subterranea (L) Verdc) grown in Southern Africa. *African Journal of Biotechnology*. 2006; 5(23).
19. Trailokya A., Srivastava A., Bhole M. and Zalte N. Calcium and Calcium Salts. *Journal of the Association of Physicians of India.* 2017; 65(1):1-2.
20. World Health Organization. *Vitamin and mineral requirements in human nutrition*. 2004; World Health Organization.
21. Ojimelukwe P. C. and Ugwuona F. U. The traditional and medicinal use of African breadfruit (Treculia africana Decne): an underutilized ethnic food of the Ibo tribe of Southeast, Nigeria. *Journal of ethnic foods*. 2021; 8(1):1-13.
22. Eneogwe O. G., Okpala O. E., Anthony W. O., Obuye F., Ibrahim I. E. and Bilkisu I. A. Nutraceutical evaluation of some selected indigenous spices found in Kogi State, Nigeria. *Fudma Journal of Sciences*. 2023; 7(5):71-79.
23. Malhotra H., Vandana A., Sharma S. and Pandey R. Phosphorus nutrition: plant growth in response to deficiency and excess. *Plant nutrients and abiotic stress tolerance*. 2018; 171- 190.
24. Minegishi S., Luft F. C., Titze J. and Kitada K. Sodium handling and interaction in numerous organs. *American journal of hypertension*. 2020; 33(8):687-694.
25. Muhammad S. S., Muhammad H. Y., Sheshe S. M., Aliyu R. M., Zubair A. A. Comparative Analysis of Proximate and Mineral Compositions of Bambara nut (Vigna subterranea) and Groundnut (Arachis-hypogea). *J Nutr Sci Res*. 2021; 6(6):1000150.
26. Georgieff M. K. Iron deficiency in pregnancy. *American journal of obstetrics and gynecology*. 2020; 223(4): 516-524.
27. Kyoung H., Lee J. J., Cho J. H., Choe J., Kang J., Lee H. and Song M. Dietary glutamic acid modulates immune responses and gut health of weaned pigs. *Animals*. 2021; 11(2):504.
28. Slominski A., Semak I., Pisarchik A., Sweatman T., Szczesniewski A. and Wortsman J. Conversion of L-tryptophan to serotonin and melatonin in human melanoma cells. *FEBS Letters.* 2002; 511(1):102–106.
29. Jyotsana N., Ta K. T. and DelGiorno K. E. The role of cystine/glutamate antiporter SLC7A11/xCT in the pathophysiology of cancer. *Frontiers in oncology*. 2022; 12:858462.
30. Clemente Plaza N., Reig García-Galbis M. and Martínez-Espinosa R. M. (2018). Effects of the Usage of l-Cysteine (l-Cys) on Human Health. *Molecules*. 23(3):575.
31. van Goudoever J. B., Vlaardingerbroek H., van den Akker C. H. de Groof F. van der Schoor S. R. Amino acids and proteins. *Nutritional Care of Preterm Infants*. 2014; 110: 49-63.
32. Verruck S., Balthazar C. F., Rocha R. S., Silva R., Esmerino E.A., Pimentel T. C., Freitas, M. Q., Silvac M. C., Gomes da Cruz A. and Prudencio E.S. Dairy foods and positive impact on the consumer's health. *Advances in food and nutrition research*. 2019; 89:95-164.
33. Chowdhury R., Steur M., Patel P. S. and Franco O. H. Individual fatty acids in cardiometabolic disease. In *Handbook of lipids in human function*. 2016; 207-318. AOCS Press.

34. Orch H., Chaachouay N., Douiri E. M., Faiz N., Zidane L., and Douira, A. Use of medicinal plants in dermato-cosmetology: An ethnobotanical study among the population of Izarène. *Jordan Journal of Pharmaceutical Sciences*. 2021; 14(3):323-340.
35. Heredia D., Green I., Klaasen J. and Rahiman F. Importance and relevance of phytochemicals present in *Galenia Africana*. *Scientifica*. 2022. https://doi.org/10.1155/2022/5793436
36. Saeed N. M., El-Demerdash E., Abdel-Rahman H. M., Algandaby M.M., Al-Abbasi F.A. and Abdel-Naim A.B. Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models. *Toxicology and applied pharmacology*. 2012; 264(1):84-93.
37. Kumar R., Madras G. and Modak J. Enzymatic synthesis of ethyl palmitate in supercritical carbon dioxide. *Industrial & engineering chemistry research*. 2004; 43(7):1568-1573.

تقييم التركيب الغذائي والكيميائي لبذور *Treculia Africana* و *Vigna subterranea l.* المجمعة من ولاية كوجي، نيجيريا

إنيوجوي أوكيتشوكو جودفري*[*1]، أوكبالا أونووديجو إيجيك[1]، أنتوني ويليام أوجونيكو[1]، إبراهيم إيزهي إستر[2]، أوبوي فيث[1]، أتومي أنتوني أوجبيديوجو[1]، عبد الله إدريس بيلكيسو[1]

[1] قسم الكيمياء، كلية العلوم، الجامعة الفيدرالية لوكوجا، نيجيريا.

[2] قسم الكيمياء الصناعية، كلية العلوم، الجامعة الفيدرالية لوكوجا، نيجيريا.

ملخص

تهدف هذه الدراسة إلى التحقيق في التركيب الغذائي لمحصولين من البقوليات غير المستغلة بشكل كافٍ، وهما بذور *Treculia africana*و *Vigna subterranea*، التي تم جمعها من ولاية كوجي، نيجيريا .حللت الدراسة تركيبها التقريبي ومحتواها المعدني وملف الأحماض الأمينية باستخدام طرق تحليلية قياسية .بالإضافة إلى ذلك، تم تحديد التركيب الكيميائي للعينة باستخدام كروماتوغرافيا الغاز-مطيافية الكتلة .أظهرت النتائج وجود فروق ذات دلالة إحصائية ($P \leq 0.05$)في عينات البقوليات .ومع ذلك، كان لبذور *V. subterranea* أقل محتوى للرطوبة 0.81 ± 12.90)٪ (بالإضافة إلى أعلى محتوى للدهون الخام ومحتوى الألياف الخام والبروتين الخام ومحتوى الرماد بنسبة 0.41 ± 15.70٪ و 0.16 ± 5.06٪ و 0.25 ± 27.86٪ و 0.50 ± 3.23٪ على التوالي .أشار التحليل العنصري بالملغ 100/جرام إلى أن العينات تحتوي على مستويات ملحوظة من المعادن الأساسية .كان لدى *T. africana* أعلى تركيزات من المغنيسيوم والفوسفور والصوديوم والبوتاسيوم والحديد بواقع 1.70 ± 190.03مجم 100/جرام و 1.60 ± 315.95مجم 100/جرام و 1.82 ± 32.61 مجم 100/جرام و 2.61 ± 1941.53مجم 100/جرام و 1.73 ± 39.50مجم 100/جرام على التوالي، بينما كان الكالسيوم (1.63 ± 58.46)أكثر وفرة في *V. subterranea* وكانت العينات غنية أيضًا بالأحماض الأمينية، وهي اللبنات الأساسية للبروتين .ومع ذلك، كان *V. subterranea* هو الأغنى بمحتوى الأحماض الأمينية، حيث كان يحتوي على ± 33.07 2.22جم 100/جم و 4.24 ± 46.01جم 100/جم، للأحماض الأمينية الأساسية وغير الأساسية، على التوالي .أظهر توصيف كروماتوغرافيا الغاز ومطياف الكتلة للتركيب الكيميائي للعينات أن حمض الميريستيك (48.1)كان الأكثر وفرة في *T. africana*، بينما كان إيثيل بالميتات (31.17)الأكثر وفرة في *V. subterranea*. بشكل عام، تشير النتائج إلى أن عينات البقوليات هذه هي مصادر غنية بالخصائص الغذائية والصيدلانية المفيدة للاستهلاك البشري.

الكلمات الدالة: ملف تعريف الأحماض الأمينية، التركيب الكيميائي، تركيز المعادن، التركيب التقريبي، *Treculia africana*، *Vigna subterranea*.

---

[*] ***المؤلف المراسل: إنيوجوي أوكيتشوكو جودفري***

godfrey.eneogwe@fulokoja.edu.ng

تاريخ استلام البحث 2024/03/16 وتاريخ قبوله للنشر 2024/06/14.

Anti-Tumorgenic Impact of Nano-Formulated Peptide HIF-Alpha Therapy by DMBA Induced Mammary Carcinoma in Rodent Type

Dharmar Manimaran[1], Namasivayam Elangovan[2] , Vasan Palanisamy*[*1]

[1]Department of Animal Nutrition, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal, Tamil Nadu, India.
[2] Department of Biotechnology, School of Biosciences, Periyar University, Salem, Tamil Nadu, India.

ABSTRACT

Over the past decade, personalized medicine has acquired considerable attention, emerging as a promising avenue for enhancing cancer treatment and therapy. Within this rapidly increasing field, the latest research introduces an innovative approach focused on a nano-formulated peptide, HIF-alpha, distinguished by its unique dual pharmacological potential. Various tumor-induced rat model has been undertaken to assess the peptide's efficacy in combatting DMBA-induced breast cancer. The findings clearly demonstrate the synthesized peptide's profound impact on various feature of tumor biology, including the proliferation of malignant cells, the synthesis of fatty acids crucial for cellular metabolism, and the regulation of lactate levels implicated in tumor progression. Histopathological analyses provide compelling evidence of the peptide's ability to established multifaceted pharmacological effects within the tumor microenvironment. Moreover, it has been demonstrated that regulatory influence on key membrane receptors, namely HER2 and EGFR, further underscores its therapeutic promise. In summary, the peptide HIF-alpha emerges as a potential landmark, offering a more efficacious therapeutic adjunct to existing medications, irrespective of the malignancy's stage. This innovative discovery holds transformative potential in reshaping conventional cancer treatment paradigms, heralding a new era of precision medicine in oncology.

Keywords: Personalized medicine, HIF-alpha, breast cancer, Peptide and Therapeutic adjuvant.

1. INTRODUCTION

Breast cancer is the most ubiquitous, persistent, leading cause of invasive fatalities and illnesses among women worldwide in developed and developing nations. These cells become altered and uncontrolled once breast cancer progresses, resulting in the formation of a tumor. The greatest mortality is driven mostly by a lack of treatment choices for late disease stages. Regardless of stage of the illness, a range of therapeutic techniques have been granted for use in traditional therapies reduce the therapeutic efficacy [1, 2, 3, 4, 5, 6].

Peptides found diverse applications in cancer treatment, including their direct use as drugs such as angiogenesis inhibitors, as well as serving as tumor-targeting agents to deliver cytotoxin drugs and radionuclides for targeted chemotherapy and radiation therapy. With their ability to bind to various receptors and participate in biochemical pathways, peptides also play a crucial role as diagnostic tools and biomarkers in understanding cancer progression [7, 8, 9].

Peptide hormone treatment and tumor-targeting drugs with radionuclides for imaging and therapy are the main sources of the peptide medications that are currently available on the market [10]. Bortezomib and mifamurtide

[*]***Corresponding author: Vasan Palanisamy***
drpvasan@yahoo.com
Received: 18/03/2024 Accepted: 14/06/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2482

 © 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

are two shorter peptide medications that deviate from the requirements [11, 12].

A significant mechanism in which cancer cells adapt to low oxygen levels in their environment is by activating hypoxia inducible factors (HIFs). These factors with two subunits: either HIF-1α or HIF-2α, which responds to oxygen levels, and HIF-1β, which remains consistently expressed. Activation of HIFs leads to the expression of genes crucial for invasion, metastasis, and resistance to treatment, contributing to increased metastasis and decreased survival rates in breast cancer patients [13]. HIF-1α specifically plays essential roles in various aspects of breast cancer progression, including metastatic site formation, angiogenesis, stem cell maintenance, metabolic changes, epithelial-mesenchymal transition (EMT), invasion, metastasis, and resistance to radiation and chemotherapy [14]. Recent studies indicate that the HIF-1 pathway regulates breast cancer metastasis through multiple mechanisms [15]. The HIF knocked mice mainly showed abnormal vascular development. In further research reported that HRE-/-Estumor produced the same level of VEGF as the VEGF-/-ES tumors indicating the role of HIF/HRE in transcription regulation of VEGF production in tumor cell [16]. Therefore, understanding these molecular and cellular processes is crucial for accurate prognosis and the development of new treatment strategies.

Chemotherapy is one among the many major therapy in order to treat cancer, for it delivers the cytotoxic agent to the cancer cell. But here again is the limitations whereas in there is the inability to deliver the appropriate amount of the drug to be directly affect the cancer without disturbing the normal cell. Other effects also noted are that the drug resistance, the altered bio distribution, and clearance of the drug also seem to be set as a common problem. Yet the process of the targeted chemotherapy and the techniques of drug delivery have now become as a powerful method to come across these problems [8, 17]. Hence forth this will allow doing a selective and an effective localization of the drug in the predefined areas thus increase the therapeutic index and reducing toxicity.

Cancer nanotechnology has been recently employed in drug design and research to encounter the aforementioned drawbacks of cancer therapies, resulting in more therapeutically efficient and safer drugs [18]. Nanotechnology is revolutionizing the pharmaceutical and medical industries, particularly in cancer research. By merging chemistry, engineering, biology, and medicine, nanotechnology enables targeted and efficient drug delivery through nanoparticles that interact with cells [19, 20]. The primary goal of nanoparticle drug delivery systems is to enhance drug effectiveness while reducing harmful side effects, thereby optimizing treatment outcomes [21].

Chitosan-based nanoparticles (ChNPs) provided appealing prospects to be explored as biodegradable and biocompatible drug delivery [22]. Chitosan-based nanoparticles (ChNPs) exhibit strong biodegradability and biocompatibility, making them promising for targeted drug delivery systems. ChNPs can deliver a variety of anticancer medicines to specific locations via passive and active targeting routes. The alteration of ChNPs attracted the researcher's interest in medication delivery to cancer cells. [23]. Accordingly, the present study was designed to evaluate the effect of nano formulated peptide HIF-alpha inhibition of HIF-alpha by introducing PCN-M04 against 7,12-dimethylbenz(a) anthracene (DMBA) induced mammary carcinoma to Sprague Dawley (SD) rats.

2. MATERIALS AND METHODS

2.1 Drugs and chemicals

Chitosan, and sodium tri poly-phosphate were procured from Sigma Aldrich (USA). All other chemicals used in this study were of analytical grade.

2.2 HIF9 Peptide Chitosan nanoparticles (PCN) Preparation

PCN was prepared by ionic cross-linking chitosan using sodium tripolyphosphate (TPP) anions. 20 mg/mL chitosan was suspended in 2%v/v aqueous acetic acid

solution at pH 5.5 with steady stirring at 10 °C (1 h).The aqueous solution comprising of chitosan: TPP ratio of 1/0.1(v/v) was incorporated in droplets to the solution and maintained in the magnetic stirrer for 3 h. 100ng/mL HIF9 (Hypoxia-Inducible Factors 9) peptide dissolved in HPLC water grade) was added and continuously swirled for 6 hours at 4 °C. The resulting solution was centrifuged at 13000rpm for 20 min at 4°C (REMI Cooling Centrifuge, Chennai -India). The resulting supernatant was collected, then peptide chitosan nanoparticles (PCN) were dried in the deep freezer (-55 °C) for further analysis [24].

2.3 Experimental animals and ethical

Female Sprague-Dawley (SD) rats (21 days old) were purchased from India's NIN (National Institute of Nutrition). The animals were reared in polypropylene cages with a 12-hour light/12-hour dark cycle, 50% humidity, and a temperature of 25 °C. Food and water were readily available. The study accompanied the ethical criteria established by Periyar University's Institutional Animal Ethical Committee, with clearance number IAEC/BT01/2016/1085/PO/OC/07/CPCSEA.

2.4 Carcinoma induction of mammary carcinoma with DMBA

Mammary cancer was developed in 36 rats by dissolving 120 mg of DMBA (Sigma Aldrich, USA) in 24 mL of olive oil and thoroughly mixing it (a yellow-colored solution is produced). The DMBA solution was administered orally to the animals through an oral feeding syringe at a dosage of 25 milligram per kilogram [25]. Following the removal of the excess DMBA, the region was cleansed and chemically inactivated using a diluted solution of sodium carbonate.

2.5 Experimental design and protocol

- Healthy SD rats were divided in six groups and each group consists of six animals:
- Group 1: Rats received Olive oil alone as control (P.O).
- Group 2: Rats in group II received that DMBA alone 25 mg/kg as negative control (P.O).
- Group 3: Rats in group III were treated with DMBA and Tamoxifen (medication used to treat breast cancer) 10 mg/kg/day (P.O) after one month of PIP (Post Induction Period) as the positive control.
- Group 4: Rat in group IV treated that with DMBA and after one month of PIP onwards 10 mg/kg/week of chitosan nanoparticles was added (I.V route).
- Group 5: Rats were treated with DMBA and after one month of PIP the animals treated with Peptide HIF9 60 µg/kg/week (I.V route).
- Group 6: Rats were treated with DMBA and after one month of PIP the animals treated with PCN-M04 5mg/kg/week (I.V route) containing of peptide HIF9 (426.18 mg of PCN-M04 contains 14 ng of Peptide HIF9).

At the end of experiment (120 days), rats were sacrificed with an excess of diethyl ether anesthesia, the breast tissues were processed for further studies.

2.6 Body weight and breast tumor weight changes

Weekly recordings of food consumption and body weight were maintained. After 20 days of DMBA injection, all rats were examined for breast tumors using palpation. The number, size, and location of tumors were noted throughout the procedure. After a histological diagnosis, the tumors' onset period was identified. Each rat was examined daily for signs of illness, and those found to be in a moribund condition were swiftly placed to death. Additionally, rats with tumors that exceeded 10% of their body weight, were larger than 15 to 20 mm in diameter, hindered normal mobility, or ulcerating during the investigation were immediately subjected to death. The tumor volume was assessed at the final stage of the experiment [26].

2.7 Hematoxylin and Eosin stain (HE stain)

Mammary tissues were surgically removed and promptly embedded in paraffin wax after being treated with 10% (v/v) formalin fixative. Five-micron thick slices were cut with a microtome (Leica, RM2135, Germany), laid out on glass slides, and cleaned with xylene. The slices

were rehydrated using a succession of ethanol solutions, rinsed with water, stained with hematoxylin, and washed with running water (20 minutes). The slides were counterstained with eosin and dehydrated using a succession of ethanol solutions. Finally, slides were mounted in DPX, photographed, and checked under a microscope (Olympus, MLXi, Japan).

2.8 Immunohistochemistry

To quantitatively measure the in vivo expression of extracellular proteins as well as the receptor occupancy of any ligand-of-interest (i.e., therapeutics or imaging agents) was an important tool for personalized medicine, including tumor detection the portions were deparaffinized and rehydrated in xylene. Immunohistochemistry for epidermal growth factor receptors (ErbB-1) was performed using the ScyTek (PolyTekTM) kit protocol. Concurrently, IHC for the tyrosine-protein receptor erbB2 (HER2) was carried out using the NovolinkTMpolymer detection system (Leica) kit in accordance with the manufacturer's protocol.

2.9 Statistical analysis

All data were presented as mean ± Standard Error (SE) of number of experiments. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using Graphpad PRISM (Version-5.01, USA) and the individual comparison were obtained by "Dunnett" or "Bonferroni" (Chapter II) comparisons. A value of $P<0.05$ was considered to indicate a significant difference between groups.

3. RESULTS

3.1 The reduction of tumor incidence

Figure 1 illustrates the initial and final body weight variations. Initially, there were no significant differences in the body weights of the control and experimental animals. However, when compared to control groups, there was a significant decrease in body weight in the experimental groups. Significant weight differences were noted across the different groups. Figures (2 and 3) indicate the mean tumor incidence and average number of tumors for each treatment group. At the end of experiments, the tumor incidence and average number of tumors among the groups observed as a consequence of Tamoxifen treated animals (Group-3) were insignificant. However, the DMBA and PCN-M04 treated rats (Group 6) exhibited a larger reduction in tumor incidence (25-30%) and tumor number (1-1.5). Groups 2 and 3 showed no change.

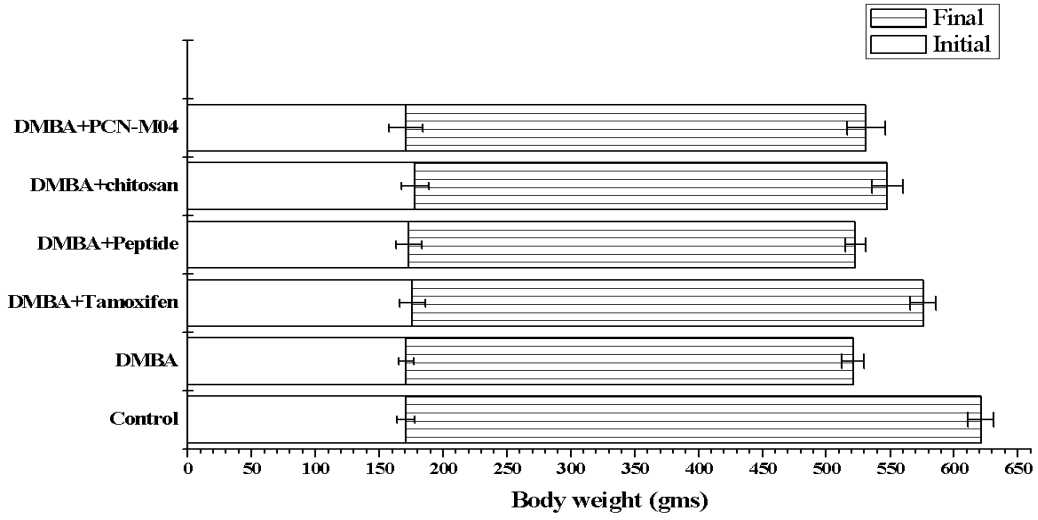


Fig.1. Variations in body weight of control and experimental animals.

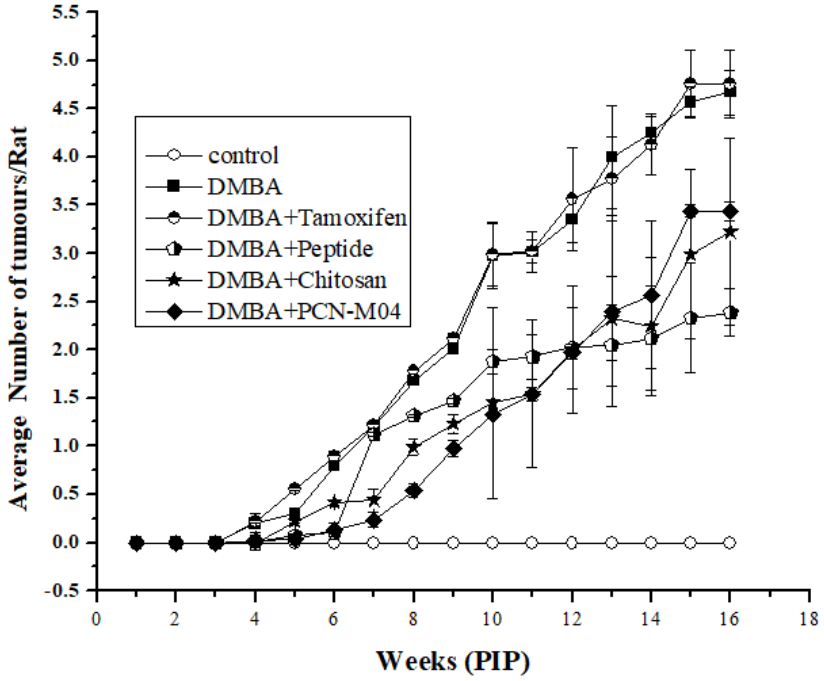


Fig.2. The incidence of rat mammary tumour growth in groups of experiments under control.

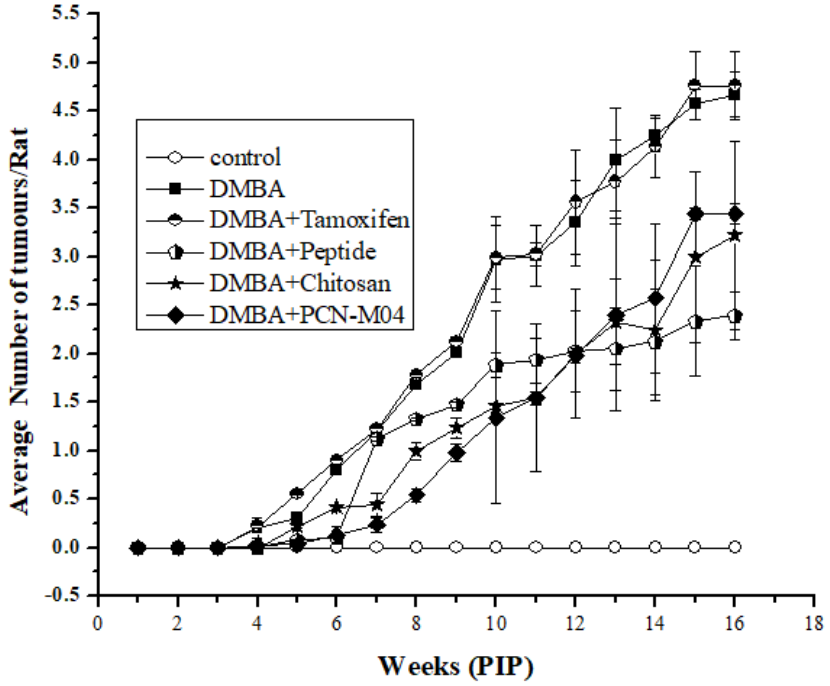


Fig.3. The normal amount of tumours observed in the experimental and control groups.

3.2. Histological Examination

Figure 4 depicts microscopic findings that occurred after treatment with PCN-M04 cells, demonstrating signs of apoptosis such as remarkable decrease in matrix, cristae, and lipid bodies. The sole DMBA treated group demonstrated endoplasmic reticulum abnormalities, aberrant nucleus organization, and mitochondrial enlargement.

3.3 Immunohistochemistry

The HER2 protein test is designated on IHC slides based on FDA scoring requirements. (Figure 5). Group 1 membrane staining reveals fewer than 10% of tumor cells (Negative). Group 4 showed faint/barely perceptible membrane staining (Negative), Group 5 and 6 showed week to moderate staining and Group 2 and 3 showed a strong and complete membrane staining with more than 10% tumor cells (Strongly Positive).

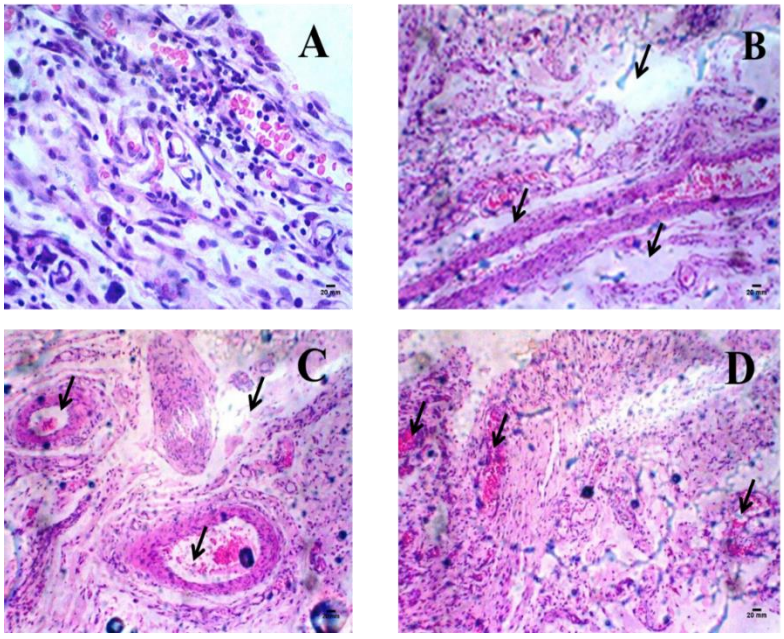


Fig.4. Breast tissue histopathology (A) Showing normal architecture (Group-1), (B) (Group-3: DMBA + Tamoxifen) Tumor section showing reactive lymphatic follicular hyperplasia with numerous mitotic feature (Group-3),(C) Section showing neoplastic cells invasive feature- (Group-2) and (D) Papillary tumor invasive feature with apoptosis (Group-5).Treatment- Group 1:Olive oil, Group 2 : DMBA, Group 3: DMBA + Tamoxifen, Group 4: DMBA + Peptide (HIF9), Group 5: DMBA + Chitosan, Group 6: DMBA + PCN –M04.

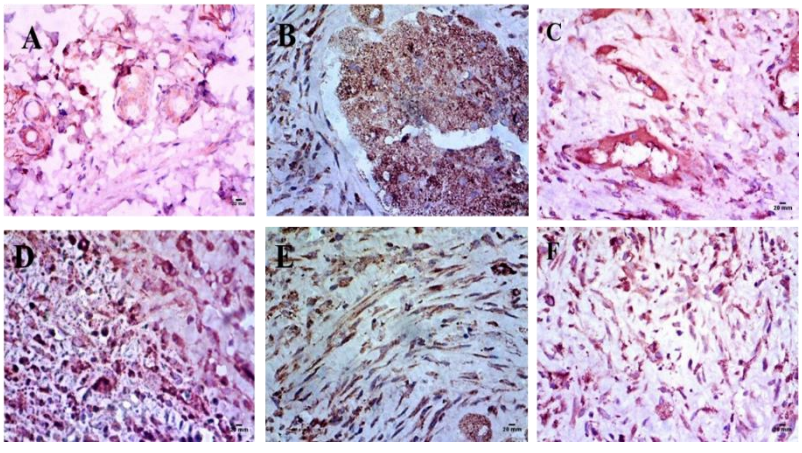
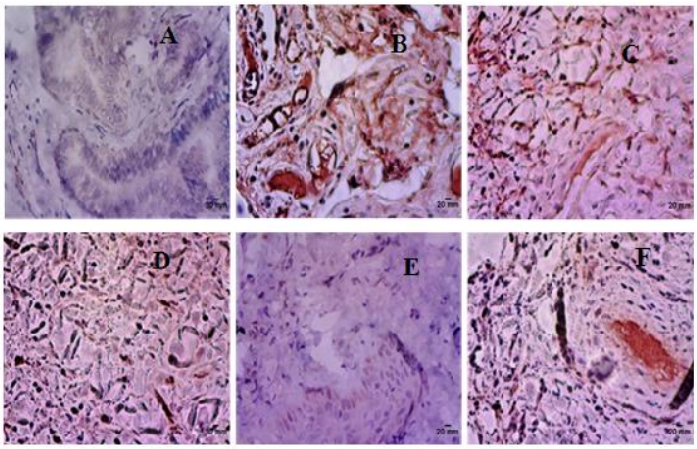


Fig.5. DAB Chromogen expression of HER2 protein in rat mammary tissues. Tissues with HER2 IHC Scores of 3+ (B & C), 2+ (E &F), 1+(D) and 0 (A) were subjected to HER2 Immunohistochemistry (IHC). Treatment- (A) Group 1: Olive oil, (B) Group 2: DMBA, (C) Group 3: DMBA + Tamoxifen, (D) Group 4: DMBA + Peptide (HIF9), (E) Group 5: DMBA + Chitosan, (F) Group 6: DMBA + PCN – M04.



. Fig.6. DAB Chromogen expression of EGFR Protein in rat mammary tissues. Score 0 by ICH (A, E),Score 3 by IHC B,D & E, Score 2 by IHC (C) .Treatment- (A) Group 1: Olive oil, (B) Group 2: DMBA, (C) Group 3: DMBA + Tamoxifen, (D) Group 4: DMBA + Peptide (HIF9), (E) Group 5: DMBA + Chitosan, (F) Group 6: DMBA + PCN – M04.

4. DISCUSSION

Nowadays, the initial stage of cancer therapy would be targeted medication delivery and finding an effective targeting agent and look for would be critical. Some of the substances which may show beneficial effect on exact targeting site that would pave the way to take forward the substance for next level of cancer therapy. DMBA is known as a site-specific cancer-causing agent and it is a lipophilic agent diversely used to study the mammary carcinogenesis. Considering this fact, the present study was planned to analyze the effect of PCN-M04 on DMBA induced mammary carcinogenesis. Further, histogenesis a therapeutic strategy is adopted in this study.

Number of studies revealed that breast cancer is infiltrated by mast cells in particular breast cancer itself exhibiting a high heterogeneity. Cancer cell might adopt in the hypoxic microenvironment, and it can be activated by HIF-α factor which act as a key factor for metastatic niche formation, angiogenesis, stem cell maintenance, metabolic reprogramming, epithelial-mesenchymal transition, invasion, metastasis and radiation therapy resistance and chemotherapy [27]. Recently, HIF-1α in human breast cancer cells inhibits primary tumor growth and lung metastasis and blocks metastatic niche formation in the lungs of mice bearing primary breast tumors [28]. The present study revealed that PCN therapy for DMBA treated rats showed reduced tumor cell proliferation was significantly decreased when compared to negative control. This phenomenon perhaps owes to the hypoxic nature happened in the tumor site on PCN-M04 received groups in our study whereas the nutritional starvation (lipids) and oxidative stress happened in the tumor site on PCN received groups.

An increased presence of mast cells has been observed in the formation of new blood vessels associated with vascular tumors, as well as various solid and blood-related cancers. However, it is particularly evident in experimental cancer development that mast cells play a significant role in tumor blood vessel formation. Mast cells located in inflamed areas more frequently contain lipid droplets, which may provide a source of amino acids for mast cell function. Thus, the role of lipids indirectly emphasizes the importance of mast cell count [29] as our findings also indicate that blocking lipid synthesis leads to some control over mast cell numbers.

Angiogenesis associated with vascular neoplasms is the major cause of solid and hematopoietic tumor as well as to increase the mast cells. Most importantly, mast cell contribution is most evident in tumor angiogenesis [21] So, the role of HIF-1 circuitously possesses the importance for mast cell count as in our results also suggest that barrier of hypoxic condition leads to nominal controlling of mast cell count.

Overexpression of HIF-1α inhibits cell death in breast

tumors and encourages the advancement of the cell cycle. Suppressing HIF-1α leads to cell cycle interruption and reduced growth. HIF-1α signaling boosts the migration of breast tumors, primarily inducing EMT to facilitate metastasis [30]. Our findings revealed that the tumor experienced higher oxidative stress because of increased energy requirements.

The findings not only strengthen the hypothesis that the activity of HIF-1α and FSN associated with breast cancer is an important molecular target for the development of cancer drugs, but they also show a close relationship between Her2 and EGFR protein, the regulation of these proteins overexpression and activation is recognized to be a significant contributing factor to their formation. The signaling molecules involved in the physiological processes that ensued from the suppression of fatty acid synthesis activity by HIF-1α and FSN were identified. Interestingly, we found in our study that group 5 and 6 showed lower levels of Her2 and EGFR proteins. This could be due to ligand-dependent hypo activation of GF receptors (GFRs) as well as loss of function of signaling cascade components like phosphatase and tensin homologue (PTEN) function [31].

5. CONCLUSION

The findings of this research offer demonstrable support for HIF-alpha peptide treatment against breast cancer, which works by preventing de novo lipogenisis. Her2 and EGFR protein control provides proof of this peptide's dual activity. This peptide can be used alone or in conjunction with current treatments to provide an efficient anti-cancer treatment.

Ethical Approval

The study accompanied the ethical criteria established by Periyar University's Institutional Animal Ethical Committee, with clearance number IAEC/BT01/2016/1085/PO/OC/07/CPCSEA.

Acknowledgments

This work was supported by The Tamil Nadu Educational Trust, Rani Seethai Hall, 6 th Floor, No.603, Annasalai, Chennai- 600 006, Tamil Nadu, India for their technical support.

Declaration of conflict

The authors declare that they have no conflicts of interest.

REFERENCES

1. Fathi M., Alami-Milani M., Geranmayeh M.H., Barar J., Erfan-Niya H. and Omidi Y. Dual thermo-and pH-sensitive injectable hydrogels of chitosan/(poly(N-isopropylacrylamide-co-itaconic acid)) for doxorubicin delivery in breast cancer. *Int. J. Biol. Macromol*. 2019; 128:957–964. doi: 10.1016/j.ijbiomac.2019.01.122.
2. Mirza Z. and Karim S. Nanoparticles-based drug delivery and gene therapy for breast cancer: Recent advancements and future challenges. *Semin. Cancer Biol.* 2021; 69:226–237. doi: 10.1016/j.semcancer.2019.10.020.
3. Richa M., Muhammad Y., Maywan H., Amira M.G., Muchtaridi M. α-Mangostin and its derivatives against estrogen receptor alpha. *J. Biomol. Struct. Dyn*. 2020;1–14. doi: 10.1080/07391102.2020.1841031.
4. Mostafa A.S., Gomaa R.M. and Elmorsy M.A. Design and synthesis of 2-phenyl benzimidazole derivatives as VEGFR-2 inhibitors with anti-breast cancer activity. *Chem. Biol. Drug Des*. 2019; 93:454–463. doi: 10.1111/cbdd.13433.

5. Ansari M.A., Thiruvengadam M., Farooqui Z., Rajakumar G., Jamal Q.M.S., Alzohairy M.A., Almatroudi A., Alomary M.N., Chung I.-M. and Al-Suhaimi E.A. Nanotechnology, in silico and endocrine-based strategy for delivering paclitaxel and miRNA: Prospects for the therapeutic management of breast cancer. *Semin. Cancer Biol*. 2019. doi: 10.1016/j.semcancer.2019.12.022.
6. Shanmuganathan R., Edison T.N.J.I., LewisOscar F., Kumar P., Shanmugam S. and Pugazhendhi A. Chitosan nanopolymers: An overview of drug delivery against cancer. *Int. J. Biol. Macromol.* 2019; 130:727–736. doi: 10.1016/j.ijbiomac.2019.02.060.
7. Borghouts C., Kunz C. and Groner B. Current strategies for the development of peptide-based anti-cancer therapeutics. *J Pept Sci*. 2005; 11(11):713–726.
8. Enback J. and Laakkonen P. Tumour-homing peptides: tools for targeting, imaging and destruction. *Biochem Soc Trans*. 2007; 35(4):780–783.
9. Zhang X.X., Eden H.S. and Chen X. Peptides in cancer nanomedicine,drug carriers,targeting ligands and protease substrates. *J Control Release*. 2012; 159:2-13.
10. Thundimadathil J. Cancer treatment using peptides: current therapies and future prospects. *Journal of amino acids*. 2012; 9(6):7-16.
11. Adams J., and Kauffman M. Development of the proteasome inhibitor Velcade™ (Bortezomib). *Cancer investigation*. 2004; 22(2):304-311.
12. Meyers P. A., Schwartz C. L., Krailo M., Kleinerman E. S., Betcher D., Bernstein M. L., and Grier H. Osteosarcoma: a randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. *Journal of clinical oncology*. 2005; 23(9):2004-2011.
13. Manimaran D., et al. Molecular insights of newly identified potential peptide inhibitors of hypoxia inducible factor 1α causing breast cancer. *J. Mol. Struct.* 2019; 1177:558–563. https://doi.org/10.1016/j.molstruc.2018.09.072.
14. Semenza G.L. Hypoxia-inducible factors: mediators ofcancer progression and targets for cancer therapy. *Trends Pharmacol Sci*. 2012; 33(4):207-214.
15. Li X., Chen Y.T., Josson S., Mukhopadhyay N.K., Kim J., Freeman M.R. and Huang W.C. MicroRNA-185 and 342 inhibit tumorigenicity and induce apoptosis through blockade of the SREBP metabolic pathway in prostate cancer cells. *PLoS One*. 2013; 8(8):e70987.
16. Chen I.T., et al. Characterization of p21Cip1/Waf1 peptide domains required for cyclin E/Cdk2 and PCNA interaction. *Oncogene*. 1996; 12(3):595–607.
17. Dorsam R.T. and Gutkind J.S. G-protein-coupled receptors and cancer. *Nat Rev Cancer*. 2007; 7(2):79–94.
18. Afzal M., Alharbi K. S., Alruwaili N. K., Al-Abassi F. A., Al-Malki A. A. L., Kazmi I., and Anwar F. Nanomedicine in treatment of breast cancer–A challenge to conventional therapy. *In Seminars in cancer biology*. 2021; (69):279-292.
19. Abu Dayyih W., Hailat M., Albtoush S., Albtoush E., Abu Dayah A., Alabbadi I. and Hamad M. F. Nanomedicine Advancements in Cancer Therapy: A Scientific Review. *Jordan Journal of Pharmaceutical Sciences*. 2014; 17(3).
20. Ibraheem L. M. and Khattabi A. M. Studying the Effect of Functional Group and Size of Silica Nanoparticles Loaded with Quercetin on their in vitro Characteristics. *Jordan Journal of Pharmaceutical Sciences*. 2022; 15(4).
21. Kzar H. H., Al-Gazally M. E. and Wtwt M. A. Everolimus loaded NPs with FOL targeting: preparation, characterization and study of its cytotoxicity action on MCF-7 breast cancer cell lines. *Jordan Journal of Pharmaceutical Sciences*. 2022; 15(1).
22. Jafernik K., Ładniak A., Blicharska E., Czarnek K., Ekiert H., Wiącek A.E. and Szopa A. Chitosan-Based Nanoparticles as Effective Drug Delivery Systems-A review. *Molecules*. 2023; 28(4):1963. doi: 10.3390/molecules28041963.

23. Herdiana Y., Wathoni N., Shamsuddin S., Joni I. M., and Muchtaridi M. Chitosan-Based Nanoparticles of Targeted Drug Delivery System in Breast Cancer Treatment. *Polymers*. 2021; *13*(11):1717. https://doi.org/10.3390/polym13111717.
24. Manimaran D., Elangovan N., Mani P., Subramanian K., Ali D., Alarifi S. and Kalirajan A. Isolongifolene-loaded chitosan nanoparticles synthesis and characterization for cancer treatment. *Scientific Reports*. 2022; 12(1):19250. https://doi.org/10.1038/s41598-022-23386-4.
25. Fukamachi K., Han B. S., Kim C. K., Takasuka N., Matsuoka Y., Matsuda E. and Tsuda H. Possible enhancing effects of atrazine and nonylphenol on 7, 12-dimethylbenz [a] anthracene-induced mammary tumor development in human c-Ha-ras proto-oncogene transgenic rats. *Cancer science*. 2004; 95(5):404-410.
26. McCormick D.L., Adamowski C.B., Fiks A. and Moon R.C. Lifetime dose-response relationships for mammary tumor induction by a single administration of Nmethyl-N-nitrosourea. *Cancer Res*. 1981; 41(5):1690-4.
27. Zhang X. X., Eden H. S. and Chen X. Peptides in cancer nanomedicine: drug carriers, targeting ligands and protease substrates. *Journal of controlled release*. 2012; 159(1):2-13.
28. Chen K. and Chen X. Integrin targeted delivery of chemotherapeutics. *Theranostics*. 2011; 1:189-200.
29. Kaliaperumal J., Padarthi P., Elangovan N., and Hari N. Anti-tumorigenic effect of nano formulated peptide pACC1 by diminishing de novo lipogenisis in DMBA induced mammary carcinoma rat model. *Biomedicine & Pharmacotherapy*. 2014; 68(6):763-773.
30. Mirzaei S., Ranjbar B., Tackallou S. H. and Aref A. R. Hypoxia inducible factor-1α (HIF-1α) in breast cancer: The crosstalk with oncogenic and onco-suppressor factors in regulation of cancer hallmarks. *Pathology, research and practice*. 2023; *248*:154676. https://doi.org/10.1016/j.prp.2023.154676.
31. Javier, Menendez J.A. and Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer*. 2007; 7:763–77.

التأثير المضاد للورم لعلاج الببتيد HIF-alpha المصاغ بالنانو بواسطة سرطان الثدي الناجم عن DMBA في نوع القوارض

دارمار مانيماران[1]، ناماسيفايام إلانجوفان [2]، فاسان بالانيسامي[1]*

[1] قسم تغذية الحيوان، كلية الطب البيطري ومعهد البحوث، جامعة تاميل نادو للطب البيطري وعلوم الحيوان، ناماكال، تاميل نادو، الهند.

[2] قسم التكنولوجيا الحيوية، كلية العلوم البيولوجية، جامعة بيريار، سالم، تاميل نادو، الهند

ملخص

على مدى العقد الماضي، اكتسب الطب الشخصي اهتمامًا كبيرًا، حيث ظهر كطريق واعد لتعزيز علاج السرطان. وفي هذا المجال المتزايد بسرعة، يقدم أحدث الأبحاث نهجًا مبتكرًا يركز على ببتيد مُصاغ على شكل نانو، HIF-alpha، يتميز بإمكاناته الدوائية المزدوجة الفريدة. تم إجراء نماذج مختلفة للفئران المستحثة بالورم لتقييم فعالية الببتيد في مكافحة سرطان الثدي الناجم عن DMBA. توضح النتائج بوضوح التأثير العميق للببتيد المُصنَّع على سمات مختلفة من بيولوجيا الورم، بما في ذلك تكاثر الخلايا الخبيثة، وتخليق الأحماض الدهنية الضرورية لعملية التمثيل الغذائي الخلوي، وتنظيم مستويات اللاكتات المتورطة في تطور الورم. توفر التحليلات النسيجية المرضية أدلة دامغة على قدرة الببتيد على إحداث تأثيرات دوائية متعددة الأوجه داخل بيئة الورم. علاوة على ذلك، فقد ثبت أن التأثير التنظيمي على مستقبلات الغشاء الرئيسية، وهي HER2 وEGFR، يؤكد بشكل أكبر على وعده العلاجي. باختصار، يبرز الببتيد HIF-alpha كمعلم بارز محتمل، حيث يقدم مكملًا علاجيًا أكثر فعالية للأدوية الحالية، بغض النظر عن مرحلة الخباثة. يحمل هذا الاكتشاف المبتكر إمكانات تحويلية في إعادة تشكيل نماذج علاج السرطان التقليدية، ويبشر بعصر جديد من الطب الدقيق في علم الأورام.

الكلمات الدالة: الطب الشخصي، HIF-alpha، سرطان الثدي، الببتيد والمكمل العلاجي المساعد.

* ***المؤلف المراسل: فاسان بالانيسامي***

drpvasan@yahoo.com

تاريخ استلام البحث 2024/03/18 وتاريخ قبوله للنشر 2024/06/14.

Phytosomes: A Cutting-Edge Platform for Phytochemicals Delivery by Enhancing Bioavailability

Smita Kumbhar*[1], Vaishnavi Jagdale[2], Manish Bhatia[3]

[1] Department of Pharmaceutical Chemistry, Sanjivani College of Pharmaceutical Education and Research (Autonomous), Kopargaon, Maharashtra, India.
[2] Department of Pharmaceutics, Amepurva Forum's Nirant Institute of Pharmacy, Solapur, India.
[3] Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Kolhapur, India.

ABSTRACT

The word "Phyto" signifies plant, while "some" is for cell. This innovative patented technology involves combining plant extracts or hydrophilic phytoconstituents with phospholipids to create lipid-suitable molecular complexes, resulting in not only enhanced absorption but also bioavailability. Extensive research has been conducted by various scientists to explore the transdermal way as an excellent method for delivering phytoconstituents. Phyto products or Phyto extracts are gaining significant consideration as dietary complements in managing inflammation, toxicity, cancer, weight loss, and various chronic degenerative conditions. Nevertheless, continuous advancements and studies are being conducted in this fieldthese products frequently encounter issues with stability and bioavailability. Once extracted, plant products become susceptible to instability and may not be suitable for passage through a biological membrane. This technique enhances the hydrophilicity of highly lipophilic drugs, manufacturing them convenient for drug delivery, and adequately enhance the lipophilicity of Phyto constituents to facilitate permeation through the bio- membrane. The use of Phytosomes for beautifying purposes has already been scientifically established. Additionally, this review offers a relative analysis of liposomes and Phytosomes, highlighting current developments in Phytosomes technology, mostly in transdermal drug delivery. Incorporation of polyphenol compounds into a self-assembled phospholipid-based delivery system, known as a Phytosomes, can significantly improve their poor oral bioavailability.

Keywords: Phytosomes, phospholipid complex, bioavailability, phytoconstituents, nanocarrier.

INTRODUCTION

Phytochemicals are natural bioactive compounds produced by plants, which react with various elements of living creatures to provide useful effects. These compounds, including phenolics, alkaloids, carbohydrates, terpenoids, and other nitrogen-containing compounds, have different structures and are categorized as phytochemicals [1-5]. Additionally, differentiations in biogenesis or biosynthetic pathways result in different kinds of phytochemicals. Only phytochemicals with active H_2 atoms, such as polyphenols, can be incorporated into the structure of plants. Polyphenols, a foremost group of plant chemicals commonly found in plant-based foods, have demonstrated potential health effects in various studies. diseases including malignance, inflammation, neuron degeneration and heart diseases, type 2 diabetes and overweight [6-10]. In fact, they consist of sugar residues attached to the hydroxyl group; On the other hand, sugar residues and aromatic carbon can form direct bonds. Polyphenols can be divided into two primary groups: flavonoids and non-flavonoids. This review centers on the

*Corresponding author: Smita Tukaram Kumbhar
smitakumbhar@gmail.com
Received: 20/03/2024 Accepted: 14/06/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2488

 © 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

usage of Phytosomes to utilize polyphenols, including their structure, research, and biological processes associated with the uptake of phytochemical-rich Phytosomes [10-17]. Because of their small size (1-100 nm) and large volume, nano-based preparations can improve therapeutic efficacy. This carrier can be utilized for the intended delivery spot in addition to its size and shape. Furthermore, a variety of nanocarriers have been investigated for delivery, including liposomes, micellar systems, metallic and polymeric nanoparticles, nanostructured lipid carriers, and nano and microemulsions. The qualities of drug release, bioavailability, and consistency can all be enhanced by these nano-based transporters. At the moment, safe and sustainable nano-based formulations are also being investigated [18-25].

Principle of Phytosomes

An ordinary extraction or polyphenolic element in a nonpolar solvent combined with a stoichiometric quantity of phospholipid results in the creation of Phytosomes [26]. The flavonoids and terpenoids present in the extract enable direct complexation with phosphatidylcholine. A lipophilic phosphatidyl and a hydrophilic choline group come together to form a bivalent phosphatidylcholine molecule. The choline portion of the phosphatidylcholine molecule attaches to phytocomponents, while the lipophilic phosphatidyl moiety's body and tail encase the choline-bound substance [26-29]. Consequently, phytoconstituents develop lipid-compatible molecular complexes with phospholipids, also referred to as Phyto-phospholipid complexes. The decreased bioavailability and absorption of polyphenolic components can be attributed to two primary reasons. Firstly, these major components are composed of molecules with multiple rings that are too bulky to be absorbed through diffusion. Secondly, flavonoid molecules, the primary constituents of polyphenols, have low solubility in lipids, which hinders their passage through the cellular membrane [30-35].

Different Methods of Preparation of Phytosomes:

Phytosomes are advanced forms of herbal formulations that increase the absorption and bioavailability of phytochemicals. These are prepared by complexing the hydrophilic phytoconstituents with hydrophobic phospholipids. Various methods can be employed to prepare phytosomes, each with unique steps and benefits. Here are a few common methods:

Solvent evaporation method: The solvent evaporation method involves combining phosphatidylcholines and active pharmaceutical ingredients (APIs) or phytochemicals on a common circular base. This mixture is heated at a constant temperature for a specific duration and dissolved in a suitable solution. The resultant complex can be obtained by evaporating the solvent under vacuum conditions [36-38].

For instance, researchers developed phytosomes containing mitomycin C (MMC) complexed with soybean phosphatidylcholine using the solvent evaporation method. They dissolved 10 mg of MMC powder and 30 mg of soy phosphatidylcholine (SPC) in 12.5 ml of tetrahydrofuran (THF). This solution was stirred in a glass pressure vessel for 4 hours at 40°C, resulting in a light magenta mixture. Subsequently, THF was removed using a rotary evaporator and rotary vacuum evaporation techniques [39-45].

This process ensures the formation of phytosomes, enhancing the bioavailability and efficacy of the active compounds like MMC.

Anti-solvent precipitation method: The anti-solvent precipitation method is widely used for Phytosome preparation, leveraging soy milk (a plant-based milk rich in nutrients) and lecithin (a natural phospholipid mixture) irradiated with dichloromethane. Afterward, N-hexane is added to the resulting precipitate for overnight desiccation in vacuum desiccators. This process incorporates Icariin (a flavonoid from Epimedium) into ICA-Phytosomes through solvent precipitation. Precisely weighed ICA and Phospholipid 90H in dichloromethane produce a concentrated solution, irradiated as per experimental

parameters. The Phytosomal extract is obtained via lyophilization for 72 hours, stored briefly in an airtight amber glass container for subsequent use. This method's efficiency and standardization are evidenced by its widespread use and detailed experimental protocols [48-53].

Lyophilization method-

Lyophilization, or freeze-drying, is vital in creating Phytosomes. It starts with a solution of the active compound and phospholipids (like Soy Phosphatidylcholine, SPC), often with a solvent like Dimethyl sulfoxide (DMSO). These form a stable complex due to their chemical affinity. The solution is frozen to solidify the complex, then subjected to lyophilization, removing the frozen solvent under vacuum. This transforms the complex into Phytosomes, which are dried and stored for use in pharmaceutical or nutraceutical products. Phytosomes, with enhanced bioavailability, are achieved through this process by improving absorption and delivery of the active compound [54-60].

The figure labeled as "Figure 1" presents a graphical depiction of the preparation process of Phytosomes.

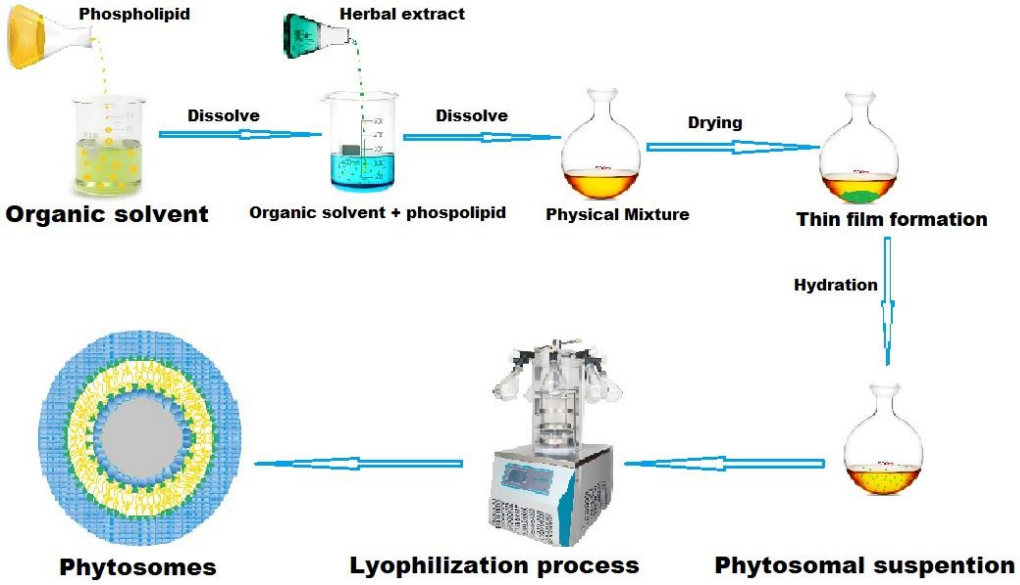


Figure1: Graphical presentation of preparation of Phytosomes.

Biological Properties of Phytosomes:

Phytosomes exhibit several biological properties that make them advantageous in various applications. Phytosomes, a technology integrating drugs, are utilized to address various medical conditions

Cardiovascular properties- Phytosomes are effective in treating peripheral vascular diseases such as Raynaud's disease and coronary artery disease. They also exhibit leuko-selective activity, reducing oxidation and oxidative stress damage to low-density lipoproteins, yielding positive results [61-63].

Anti-inflammatory properties- Phytosomes complex shows better anti-inflammatory activity than complex plant extract. Croton oil-coated dermatitis was used to evaluate the anti-inflammatory activity of the Phytosome complex in animals involving tissue and blood vessel damage. Glycyrrhizin acid, a potent anti-inflammatory agent, contributed to these beneficial outcomes [64-66].

Anti-ageing property- Phytosomes as a delivery system offers interesting applications and opens new

possibilities for the use of APIs in cosmetics [67]. G. biloba Phytosomes has been studied for the treatment of skin aging related to superficial capillary circulation. Ginkgo biloba extract is commonly taken orally to enhance peripheral circulation, while phospholipid complexes have been discovered to enhance skin microcirculation when applied topically. Microcirculation associated with dystrophic changes of the epidermis and dermis is activated in ameliorative skin aging [68-70].

Hepatoprotective property- Milk thistle (Silybum marianum) fruit flavonoids show hepatoprotective properties. Silymarin has been shown to be effective in the treatment of various types of liver diseases, including hepatitis, cirrhosis, fatty liver infiltration (chemical and alcoholic fatty liver), and inflammation of the biliary tract [71-74].

Anti-cancer property- Formulations containing Phytosomes have important pharmacological benefits, such as anti-inflammatory, antioxidant, and neuroprotective properties, and can improve the penetration and bioavailability of phytoconstituents. Although it has been studied as an anticancer agent in the treatment of many types of tumours [75-79].

Physicochemical Properties:

The physicochemical properties of Phytosomes encompass a range of characteristics:

1. The stoichiometric number of phospholipids is mixed with a standard Phyto extract as a substrate to form Phytosomes. The reaction between the phospholipid and the substrate involves the formation of H_2 bonds between the charged head of the phospholipid and the polar function of the substrate [80-83].
2. When the Phytosomes is disclosed to water, it forms micelles like liposomes, and photon correlation spectroscopy (PCS) shows that the Phytosome has this liposome structure [84].
3. Phytosomes vary in size from 51 nm to several 100 meters [85].
4. Using H1 NMR and C13 NMR data, ~~it was found that~~ fatty chains emit similar signals in free and complexed phospholipids. This suggests that the API is placed in a long aliphatic chain, creating a lipophilic envelope [86-88].

Characterization of Phytosomes-

Spectroscopy- Complex and molecular reactions of phytoconstituents and phosphatidylcholine in solution were studied by 1H-NMR, 13C-NMR, P-NMR, and Infrared spectroscopy. Complex development is related with changes in the chemical shift and line broadening of several characteristic signals in the nuclear magnetic resonance spectrum as well as the appearance of non-correlated IR spectrum [89-93].

Zeta potential- Determining the charge of Phytosomes in emulsions, which can be negative, positive, or neutral based on composition. A zeta potential greater or less than 30 mV indicates stability [94-98].

Thermal Analysis (TGA/DSC))- Determination and measurement of temperature effects such as fusion, solid-solid transition, glass transition, solvent loss and dispersion can be utilized to characterize solid Phytosome [99,100].

Entrapment efficiency- Assessing drug entrapment using ultracentrifugation and calculating efficiency (%) based on actual vs. theoretical drug amounts [101].

Particle size- Particle size and zeta potential are significant complex properties related to stability and reproducibility. Typical particle sizes of phospholipid complexes vary from 50 to 100 nm [102,103].

Advantages and Disadvatages of Phytosomes**:** Phytosomes have enhanced bioavailability, better absorption, and controlled release compared to traditional formulations, making them beneficial in pharmaceutical and cosmetic fields. They improve the solubility of poorly water-soluble compounds, expanding their applicability to various drugs and herbal extracts [104-110].

However, Phytosomes are complex and costly to prepare, requiring specialized equipment and expertise. Their stability over time can also be a concern, impacting

quality and shelf-life. Despite these challenges, Phytosomes remain valuable for enhancing drug delivery and efficacy, albeit requiring careful consideration of cost-effectiveness and stability [111].

Dosage Form of Phytosomes-

1. **Soft gelatin capsule**-These types of Phytosomes are developed in the form of heterogeneous mixtures (suspensions) with phytoconstituents as a dispersed phase such as vegetable oil or semi-synthetic oil as a dispersing media and are used to make soft gelatin phytosomes capsules for oral drug delivery e.g.- Curcumin Phyto some [112,114].
2. **Hard gelatin capsule**- Phytosomes can be used directly in the volumetric process. In powder form, it can be poured into hard gelatin capsules. For low density Phytosomes, the capsule size should not exceed 300 mg and should be zero size [115].
3. **Tablet**- Phyto-phospholipid complex powder ideal? cannot have better technological properties due to its potential viscosity, flowability and low apparent density. When the direct compression process is used for the material, it must be diluted by 60-70% of the binder and its physical and chemical properties must be optimized. For primary processing, a dry granulation process may be optimal to achieve dose uniformity and optimal bioavailability. To put it differently, it is advisable to steer clear of the wet granulation process because water and heat (utilized for granulation/drying) have an adverse impact on the constancy of the phospholipid complex [116-120].
4. **Topical dosage form**- Phyto-phospholipid complexes can be manufactured mainly in the form of creams, gel, or ointments. The innovative process, which includes a complex of Phytosomes, dispersed in a minor amount of oily phase and added to the emulsion has been formed at a low temperature (not higher than 40 °C). If the outer phase is an aqueous phase, the Phyto some complex can dissolve into the aqueous phase and add another formulation after 40°C [121].

Challenges in Developing Phytosome Products: —

Phytosomes have been developed as a potential nanocarrier delivery system. However, it is a lengthy method from product synthesis to successful marketing. As a substitute of all supplements, only phytosomal products were presented to the market [122]. After emerging an effective formulation, proving nontoxicity is the main obstacle to bringing Phytosomes to market. Phytosomes are an impartial biological system, so their entry into the body continues without problems related to safety or immunological effects. Nevertheless, prior to being marketed, it is imperative to thoroughly investigate various factors including bioaccumulation, biocompatibility, metabolism, and excretion due to the minuscule dimensions of these particles [123]. Curcumin was successfully synthesized in Phytosomes for intravenous administration in mice and was found to accumulate more in spleen tissue. Another factor to be evaluated is the passive targeting of healthy cells by Phytosomes binding to biological membranes. Therefore, the precise biological effects should be investigated in well-structured pre- clinical trials. Several studies in this area have discovered the biological protection of Phytosomes. After creating the Phytosomes, pharmacokinetics (pk) and pharmacodynamics (pd) test should be performed in animals and humans to confirm its advantage over purity of phytocomponents. Identifying the finest dosage form to maximize absorption and potency of the Phytosomes is another step for commercialization.

Additional task is the large-scale manufacturing of Phytosomes. However, the properties of the product must be maintained during upscaling. This relates to laboratory protocol practices in production settings. However, the method to produce some types of Phytosomes is sometimes easy compared to pH-sensitive Phytosomes; Low physical and chemical stability makes commercial production difficult. Similar to pharmaceuticals, Phytosomes must be able to reproduce and their properties must be tested over time. Popularity is another reason for

effective product marketing. Taken together, the biocompatibility, cost and safety of phytochemicals have influenced people to such treatments in recent years. Furthermore, due to the simple production method and the ease of commercializing phytosomal technology on a business scale of Phytosomes is a fast process [124].

Structure of Phyto-Phospholipid Complexes-

According to Bombard Elli's theory, phospholipids can combine stoichiometrically with APIs that are isolated from plants to form phospholipid complexes. This preliminary explanation of Phyto-phospholipid complexes has been questioned in light of later research. We have suggested a recent list of the four necessary elements depend on the literature: solvents, Phyto-APIs, phospholipids, and the stoichiometric proportion tangled in the creation of Phyto-phospholipid complexes.

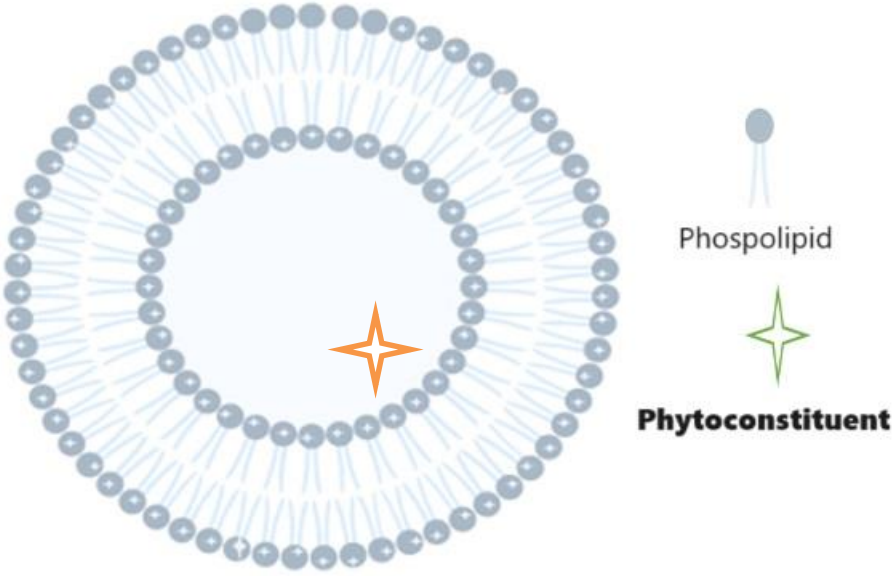


Figure 2: Phytosomes as Carriers for Herbal Drugs

Plant seeds and egg yolk are rich in phospholipids. Phospholipids made in an industrial setting are already accessible. Depending on the backbone, phospholipids can be classified as Glycerophospholipids or sphingomyelins (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylinositol (PI), and phosphatidylglycerol (PG) are additional glycerophospholipids.

The main phospholipids used to manufacture complexes with a water-soluble head group and two lipid soluble hydrocarbon chains are PC, PE, and PS. The most often utilized phospholipid among these is PC, which is utilized to create phospholipid complexes. the PC is structured as follows. One of PC's advantages is its amphipathic qualities, which allow it to dissolve somewhat in lipid and water-based environments. Furthermore, PC has strong biocompatibility and minimal toxicity because it is a necessary part of cell membranes. PC molecules have been shown to demonstrate clinical benefits in the treatment of and exhibit hepatoprotective properties include hepatocirrhosis, fatty liver, and hepatitis.

Phyto-active Constituents-

Rather than in vivo activities, scientists frequently designate the APIs of plant extracts based on strong in vitro pharmacological effects. These substances are mostly polyphenols. The medications made of polyphenols are

displayed below. cirantin is one of the physiologically active elements of plants that has a preference for the water phase and is impotent to cross bio-membranes. However, some, like rutin and curcumin, are very lipid soluble and not able to dissolve in aqueous phase and in gastrointestinal fluids. In addition to increasing lipophilic solubility in water phase, Phyto-phospholipid complexes to increase hydrophilic polyphenols' ability to pass across membranes from a water phase. Moreover, the formation of complexes can shield polyphenols from degradation [124].

Solvents-

Solvents of different kinds have been effectively explored. Ethanol is a common and helpful solvent that leaves behind phospholipid complexes when the yield is high enough. less damage and leaves less residue behind. Certain liposomal drug complexes function when H_2O or buffer solution is present, allowing the Phytosomes to be mixed with a solvent that has a lower dielectric constants technique has been implemented in numerous studies recently to control the morphology, size. One of the SCF technologies that is showing promise for manufacturing micro and sub micro particles with regulated sizes and size distributions is the supercritical anti solvent method (SAS) [124].

Stoichiometric Ratio of Active Constituents and Phospholipids-

The stoichiometric ratio of active constituents to phospholipids varies, with ratios ranging from 0.4:1 to 1.9:1 commonly used for phospholipid complexes. While a 1:1 ratio is often considered optimal, other ratios like 1:10, 1:15, and even 3:1 have been tested, with some studies showing better results at non-1:1 ratios. Therefore, a 1:1 ratio may not always be the most effective when creating phospholipid compounds.

Reaction Between Active Constituents and Phospholipids-

The chemical interaction between flavonoids and phospholipids was discovered in 1989 by Bombard Elli, resolving previous debates on Phyto-phospholipid complex formation. Molecular analysis revealed that the hydrogen bond formation between the polar head and polar functionalities of the Phyto API drives the phospholipid-API interaction in these complexes. When combined, phospholipid complexes with free active constituents show enhanced absorption and higher bioavailability. This discovery has sparked increased interest in Phytosome creation, particularly in the pharmaceutical industry where phytosomal formulations are extensively used. Marketed products based on Phytosomes, such as curcumin Phytosomes for antioxidants and green tea leaf Phytosomes for weight management, exemplify this trend [123, 124].

Factors influencing Phospholipid Complexes include solvent proportion of API, reaction temperature, and duration.

Complexation Rate of Phospholipid Complexes-

The complexation rate of phospholipid complexes is a critical measure in drug screening, determined by various factors such as the stoichiometric ratio of active components, temperature, duration, drug concentration, and solvent-to-phospholipid ratio. The productivity (%) of the complexes can be calculated using the formula: Productivity (%) = $[(x-y)/x] \times 100\%$, where "x" represents the initial mass of the active constituent, "y" is the mass of the free active component, and "(x – y)" indicates the mass or content of the phospholipid complexes. UV spectrophotometry or HPLC methods are commonly used to assess the yield of these complexes.

Industrial application of Phytosomes-

Phytosomes, formed by combining phospholipids with plant extracts like glycosides, flavonoids, and terpenoids, are advanced liposomes with excellent skin penetration and lipid content, making them ideal carriers and skin-nourishing agents in herbal cosmetics. Their chemical bonds with α-phosphatidylcholine and plant constituents enhance stability and absorption, reducing dosage requirements. Commercial products like Ginkgo biloba terpenes, known for their anti-inflammatory and calming

effects, are available in Phytosome-based topical formulations. Italian pharmaceutical companies are leading in Phytosome products, using standard plant extracts like polyphenols and terpenoids to address various health issues. Manufacturers like Natural Factors (Canada) and Nature's Herbs (USA) offer a range of Phytosome formulations with details on source, dosage, and pharmacological activity. Table 1 presents the Marketed Products of Phytosomes, while Table 2 showcases Patented Phytosomes Formulations, and Table 3 details Phytosomes and Their Activity.

Table1: Marketed Products of Phytosomes

No	**Marketed Phytosomes**	**Sources**	**Biological Activity**	**Application of technology**
1.	Silybin Phytosomes	Silybum marianum	Hepatoprotective and Antioxidant	Increase in therapeutic effect
2.	Ginkgo Phytosomes	Ginkgo biloba	heart protective, anti-asthmatic and anti-glycaemic	Increase hepatoprotective effect
3.	Ginseng Phytosomes	Panax ginseng	Nutraceutical, Immunomodulator	Increase absorption
4.	Green tea Phytosomes	Camellia sinensis	Nutraceutical, antioxidant, Anticancer	Increase absorption

Table 2: Patented Phytosomes Formulation

Serial Number	**Patent Name**	**Description of Innovation**	**Patent Number**
1.	Olive fruit phospholipid complex or extract composition deposit	Increase bioavailability	WO/2007/118631
2.	treatment of aging and skin damage by cosmetic preparation	topical treatment by cosmetic and dermatological composition	EP/1640041
3.	plant-based antioxidant preparation transfer	For treatment of adiposity problems	US/6756065
4.	wound healing by thymosin beta-4	Thymosin beta-4 for skin and wound healing	US/2007/0015698

Table 3: Phytosomes and Their Activity

Phytosomes	**Phytosomes Activity**
Curcumin	Wound Healing
Rutin	Anti-carcinogenic, Vaso protective
Houttuynia cordata	Anti-ageing, Anti-inflammatory
Silymarin	Free Radical scavenging
Grape seed extract	Reduce oxidative stress
Green tea	Anti-oxidant

Conclusion- Phytosomes is an advanced form of herbal drug administration technology that can control the disadvantages associated with regular dosage forms, such as bioavailability issues, dose omission, and site-specific delivery. Initially, this concept was used by the cosmetics industry. Today, its importance as a plant carrier has emerged in the pharmaceutical, pharmaceutical and preservative industries. Although many herbal medicines eliminate the root cause of the disease, it is hard to achieve therapeutic effectiveness. Therefore, the Phyto-

phospholipid complex of small size delivers active action directly to the site of need and acts with minimal side effects associated with minimal synthetic drugs. Phospholipids form complexes with Phyto active substances and keep active by forming bonds, thereby feeding lipophilic and hydrophilic components to the membrane. The formulation method for Phyto some is easy and can be quickly scaled up to commercial scale. It is commonly employed in both oral and topical medications, enhancing stability and improving their efficacy as narcotics.

Competing Interests: The authors have no competing interests to declare that are relevant to the content of this article.

Funding: No funds, grants, or other support was received.

Ethics Approval: Ethics Committee has confirmed that no ethical approval is required.

Consent for publication: The authors have approved Consent for publication.

Data Availability: All data generated or analysed during this study are included in this published article.

REFERENCES

1. Barani M., Sangiovanni E. and Angarano M. Phytosomes as Innovative Delivery Systems for Phytochemicals: A Comprehensive Review of Literature. *Int J Nanomedicine*. 2021; 16: 6983-7022.
2. Jadhav N., Nadaf S., Lohar D., Ghagare P. and Powar T. Phytochemicals Formulated as Nanoparticles: Inventions, Recent Patents and Future Prospects. *Recent Pat Drug Deliv Formul*. 2017; 11(3): 173-186.
3. Shriram R., Moin A., Alotaibi H., et al. Phytosomes as a Plausible Nano-Delivery System for Enhanced Oral Bioavailability and Improved Hepatoprotective Activity of Silymarin. *Pharmaceuticals (Basel)*. 2022; 15(7): 790.
4. Nuchuchua O., Inpan R., Srinuanchai W., et al. Phytosome Supplements for Delivering *Gymnema inodorum* Phytonutrients to Prevent Inflammation in Macrophages and Insulin Resistance in Adipocytes. *Foods*. 2023; 12(11): 2257.
5. Semalty A., Semalty M., Rawat M., Franceschi F. Supramolecular phospholipids-polyphenolics interactions: the PHYTOSOME strategy to improve the bioavailability of phytochemicals. *Fitoterapia*. 2010; 81(5): 306-314.
6. Gaikwad S., Morade Y., Kothule A., Kshirsagar S., Laddha U. and Salunkhe K. Overview of phytosomes in treating cancer: Advancement, challenges, and future outlook. *Heliyon*. 2023; 9(6): e16561.
7. Bibi Z. and Jahan N. Synthesis and biological evaluation of Ellettaria cardamomum (Cardamom) Phytosomes. *Pak J Pharm Sci*. 2020; 33(5): 2121-2129.
8. Islam N., Irfan M., Hussain T., et al. Piperine phytosomes for bioavailability enhancement of domperidone. *J Liposome Res*. 2022; 32(2): 172-180.
9. Mirzaei H., Shakeri A., Rashidi B., Jalili A., Banikazemi Z. and Sahebkar A. Phytosomal curcumin: A review of pharmacokinetic, experimental and clinical studies. *Biomed Pharmacother*. 2017; 85: 102-112.
10. Pandey R., Bhairam M., Shukla S. and Gidwani B. Colloidal and vesicular delivery system for herbal bioactive constituents. *Daru*. 2021; 29(2): 415-438.
11. Teja P., Mithiya J., Kate A., Bairwa K. and Chauthe S. Herbal nanomedicines: Recent advancements, challenges, opportunities and regulatory overview. *Phytomedicine*. 2022; 96: 153890.

12. Chouhan N., Mittal V., Kaushik D., Khatkar A. and Raina M. Self-emulsifying drug delivery system (SEDDS) for phytoconstituents: a review. *Curr Drug Deliv*. 2015; 12(2): 244-253.
13. Rahman M., Beg S., Verma A., et al. Phytoconstituents as pharmacotherapeutics in rheumatoid arthritis: challenges and scope of nano/submicromedicine in its effective delivery. *J Pharm Pharmacol*. 2017; 69(1): 1-14.
14. Kesarwani K., Gupta R. and Mukerjee A. Bioavailability enhancers of herbal origin: an overview. *Asian Pac J Trop Biomed*. 2013; 3(4): 253-266.
15. Mahomoodally M., Sadeer N., Edoo M. and Venugopala K.N. The Potential Application of Novel Drug Delivery Systems for Phytopharmaceuticals and Natural Extracts - Current Status and Future Perspectives. *Mini Rev Med Chem*. 2021; 21(18): 2731-2746.
16. Saraf S., Gupta A., Alexander A., Khan J., Jangde M. and Saraf S. Advancements and Avenues in Nanophytomedicines for Better Pharmacological Responses. *J Nanosci Nanotechnol*. 2015; 15(6): 4070-4079.
17. Alkholifi F., Alam A., Foudah A. I., and Yusufoglu H. S. Phospholipid-Based Topical Nano-Hydrogel of Mangiferin: Enhanced Topical Delivery and Improved Dermatokinetics. *Gels (Basel, Switzerland)*. 2023; *9*(3): 178.
18. Jahangir M., Anand C., Muheem A., et al. Nano Phytomedicine Based Delivery System for CNS Disease. *Curr Drug Metab*. 2020; 21(9): 661-673.
19. Mughees M. and Wajid S. Herbal Based Polymeric Nanoparticles as a Therapeutic Remedy for Breast Cancer. *Anticancer Agents Med Chem*. 2021; 21(4): 433-444.
20. Abu Dayyih W., Hailat M., Albtoush S., Albtoush E., Abu Dayah A., Alabbadi I., and Hamad M. F. Nanomedicine Advancements in Cancer Therapy: A Scientific Review. *Jordan Journal of Pharmaceutical Sciences*. 2024; *17*(3), 506–529.
21. Ben-Shabat S., Yarmolinsky L., Porat D. and Dahan A. Antiviral effect of phytochemicals from medicinal plants: Applications and drug delivery strategies. *Drug Deliv Transl Res*. 2020; 10(2): 354-367.
22. Mohapatra D., Agrawal A. and Sahu A. Exploring the potential of solid dispersion for improving solubility, dissolution & bioavailability of herbal extracts, enriched fractions, and bioactives. *J Microencapsul*. 2021; 38(7-8): 594-612.
23. Barkat M., Goyal A., Barkat H., Salauddin M., Pottoo F. and Anwer E. Herbal Medicine: Clinical Perspective and Regulatory Status. *Comb Chem High Throughput Screen*. 2021; 24(10): 1573-1582.
24. Nuchuchua O., Inpan R., Srinuanchai W., et al. Phytosome Supplements for Delivering *Gymnema inodorum* Phytonutrients to Prevent Inflammation in Macrophages and Insulin Resistance in Adipocytes. *Foods*. 2023; 12(11): 2257.
25. Yadav N., Parveen S. and Banerjee M. Potential of nano-phytochemicals in cervical cancer therapy. *Clin Chim Acta*. 2020; 505: 60-72.
26. Chen R., Chavda V., Patel A. and Chen Z. Phytochemical Delivery Through Transferosome (Phytosome): An Advanced Transdermal Drug Delivery for Complementary Medicines. *Front Pharmacol*. 2022; 13: 850-862.
27. Rathee S. and Kamboj A. Optimization and development of antidiabetic phytosomes by the Box-Behnken design. *J Liposome Res*. 2018; 28(2): 161-172.
28. Matias D., Rijo P. and Reis C. Phytosomes as Biocompatible Carriers of Natural Drugs. *Curr Med Chem*. 2017; 24(6): 568-589.
29. Shoeibi A., Karimi E., Zareian M. and Oskoueian E. Enhancing Healthcare Outcomes and Modulating Apoptosis- and Antioxidant-Related Genes through the Nano-Phytosomal Delivery of Phenolics Extracted from *Allium ampeloprasum*. *Genes (Basel)*. 2023; 14(8): 1547.

30. Das A., Adhikari S., Deka D., et al. An Updated Review on the Role of Nanoformulated Phytochemicals in Colorectal Cancer. *Medicina (Kaunas)*. 2023; 59(4): 685.
31. Zhang J., Tang Q., Xu X. and Li N. Development and evaluation of a novel phytosome-loaded chitosan microsphere system for curcumin delivery. *Int J Pharm*. 2013; 448(1): 168-174.
32. Janjua K., Shehzad A., Shahzad R., Islam S. and Islam M. Nanocurcumin: A Double-Edged Sword for Microcancers. *Curr Pharm Des*. 2020; 26(45): 5783-5792.
33. Goktas Z., Zu Y., Abbasi M., et al. Recent Advances in Nanoencapsulation of Phytochemicals to Combat Obesity and Its Comorbidities. *J Agric Food Chem*. 2020; 68(31): 8119-8131.
34. Jamwal R. Bioavailable curcumin formulations: A review of pharmacokinetic studies in healthy volunteers [published correction appears in *J Integr Med*. 2019 Jul; 17(4): 310. *J Integr Med*. 2018; 16(6): 367-374.
35. Dewanjee S., Chakraborty P., Mukherjee B. and De Feo V. Plant-Based Antidiabetic Nanoformulations: The Emerging Paradigm for Effective Therapy. *Int J Mol Sci*. 2020; 21(6): 2217.
36. Singh V., Arora D., Ansari M. and Sharma P. Phytochemicals based chemopreventive and chemotherapeutic strategies and modern technologies to overcome limitations for better clinical applications. *Phytother Res*. 2019; 33(12): 3064-3089.
37. Kim M., Kim S. and Yang WM. Mechanisms of action of phytochemicals from medicinal herbs in the treatment of Alzheimer's disease. *Planta Med*. 2014; 80(15): 1249-1258.
38. Ahmad S. and Hafeez A. Formulation and Development of Curcumin-Piperine-Loaded S-SNEDDS for the Treatment of Alzheimer's Disease. *Mol Neurobiol*. 2023; 60(2): 1067-1082.
39. Mahjoob M. and Stochaj U. Curcumin nanoformulations to combat aging-related diseases. *Ageing Res Rev*. 2021; 69: 101364.
40. Nguyen M., Yu H., Kiew T. and Hadinoto K. Cost-effective alternative to nano-encapsulation: Amorphous curcumin-chitosan nanoparticle complex exhibiting high payload and supersaturation generation. *Eur J Pharm Biopharm*. 2015; 96: 1-10.
41. Baradaran S., Hajizadeh A., Khanjani S. and Moradi-Kor N. Protective Effects of Curcumin and its Nano-Phytosome on Carrageenan-Induced Inflammation in Mice Model: Behavioral and Biochemical Responses. *J Inflamm Res*. 2020; 13: 45-51.
42. Nandhini S., and Ilango K. Development and characterization of a nano-drug delivery system containing vasaka phospholipid complex to improve bioavailability using quality by design approach. *Res Pharm Sci*. 2020; 16(1): 103-117.
43. Mahmood T., Al-Samydai A., Sulaibi M., et al. Development of Pegylated Nano-Phytosome Formulation with Oleuropein and Rutin to Compare Anti-Colonic Cancer Activity with Olea Europaea Leaves Extract. *Chem Biodivers*. 2023; 20(8): e202300534.
44. Prabha J., Kumar M., Kumar D., Chopra S. and Bhatia A. Nano-platform Strategies of Herbal Components for the management of Rheumatoid Arthritis: A Review on the Battle for Next-Generation Formulations. *Curr Drug Deliv*.
45. Chen C., Wang J., Sun M., Li J. and Wang H. Toward the next-generation phyto-nanomedicines: cell-derived nanovesicles (CDNs) for natural product delivery. *Biomed Pharmacother*. 2022; 145: 112416.
46. Attar E., Chaudhari V., Deokar C., Dyawanapelly S. and Devarajan P. Nano Drug Delivery Strategies for an Oral Bioenhanced Quercetin Formulation. *Eur J Drug Metab Pharmacokinet*. 2023; 48(5): 495-514.
47. Yu Z., Liu X., Chen H. and Zhu L. Naringenin-Loaded Dipalmitoylphosphatidylcholine Phytosome Dry Powders for Inhaled Treatment of Acute Lung Injury. *J Aerosol Med Pulm Drug Deliv*. 2020; 33(4): 194-204.

48. Nashaat D., Elsabahy M., Hassanein K., El-Gindy G. and Ibrahim E.H. Development and in vivo evaluation of therapeutic phytosomes for alleviation of rheumatoid arthritis. *Int J Pharm*. 2023; 644: 123-332.
49. Tafish A., El-Sherbiny M., Al-Karmalawy A., Soliman O. and Saleh N. Carvacrol-Loaded Phytosomes for Enhanced Wound Healing: Molecular Docking, Formulation, DoE-Aided Optimization, and in vitro/in vivo Evaluation. *Int J Nanomedicine*. 2023; 18: 5749-5780.
50. Salman U.l., Ahmed M.B., Mazhar U.l., Shehzad A. and Lee Y. Switching from Conventional to Nano-natural Phytochemicals to Prevent and Treat Cancers: Special Emphasis on Resveratrol. *Curr Pharm Des*. 2019; 25(34): 3620-3632.
51. Yadav N., Mudgal D., Anand R., Jindal S. and Mishra V. Recent development in nanoencapsulation and delivery of natural bioactives through chitosan scaffolds for various biological applications. *Int J Biol Macromol*. 2022; 220: 537-572.
52. Behuria H., Biswal B. and Sahu S. Electroformation of liposomes and phytosomes using copper electrode. *J Liposome Res*. 2021; 31(3): 255-266.
53. Smeriglio A., Denaro M. and Trombetta D. Dietary Phytochemicals and Endrocrine-related Activities: An Update. *Mini Rev Med Chem*. 2018; 18(16): 1382-1397.
54. Suvarna V., Bore B., Bhawar C. and Mallya R. Complexation of phytochemicals with cyclodextrins and their derivatives- an update. *Biomed Pharmacother*. 2022; 149: 112862.
55. Tiwari R., Tiwari G., Sharma S. amd Ramachandran V. An Exploration of Herbal Extracts Loaded Phyto-phospholipid Complexes (Phytosomes) Against Polycystic Ovarian Syndrome: Formulation Considerations. *Pharm Nanotechnol*. 2023; 11(1): 44-55.
56. Kim S. and Imm J. The Effect of Chrysin-Loaded Phytosomes on Insulin Resistance and Blood Sugar Control in Type 2 Diabetic db/db Mice. *Molecules*. 2020; 25(23): 5503.
57. Alshahrani S. Optimization and Characterization of Cuscuta reflexa Extract Loaded Phytosomes by the Box-Behnken Design to Improve the Oral Bioavailability. *J Oleo Sci*. 2022; 71(5): 671-683.
58. Upaganlawar A., Polshettiwar S., Raut S., Tagalpallewar A. and Pande V. Effective Cancer Management: Inimitable Role of Phytochemical Based Nano-Formulations. *Curr Drug Metab*. 2022; 23(11): 869-881.
59. Babazadeh A., Zeinali M. and Hamishehkar H. Nano-Phytosome: A Developing Platform for Herbal Anti-Cancer Agents in Cancer Therapy. *Curr Drug Targets*. 2018; 19(2): 170-180.
60. Cao X., Fu M., Wang L., et al. Oral bioavailability of silymarin formulated as a novel 3-day delivery system based on porous silica nanoparticles. *Acta Biomater*. 2012; 8(6): 2104-2112.
61. Ahmad S., Khan J., Kausar T., et al. Preparation, Characterization and Evaluation of Flavonolignan Silymarin Effervescent Floating Matrix Tablets for Enhanced Oral Bioavailability. *Molecules*. 2023; 28(6): 2606.
62. Wang S., Su R., Nie S., et al. Application of nanotechnology in improving bioavailability and bioactivity of diet-derived phytochemicals. *J Nutr Biochem*. 2014; 25(4): 363-376.
63. D'Arcy M.S. A review of the chemopreventative and chemotherapeutic properties of the phytochemicals berberine, resveratrol and curcumin, and their influence on cell death via the pathways of apoptosis and autophagy. *Cell Biol Int*. 2020; 44(9): 1781-1791.
64. Gupta M., Sansare V., Shrivastava B., Jadhav S. and Gurav P. Comprehensive review on use of phospholipid-based vesicles for phytoactive delivery. *J Liposome Res*. 2022; 32(3): 211-223.
65. Zhang Z., Li X, Sang S., et al. A review of nanostructured delivery systems for the encapsulation, protection, and delivery of silymarin: An emerging nutraceutical. *Food Res Int*. 2022; 156: 111314.

66. Casula E., Letizia Manca M. and Manconi M. An integrative review on the uses of plant-derived bioactives formulated in conventional and innovative dosage forms for the local treatment of damaged nasal cavity. *Int J Pharm*. 2021; 610: 121-229.
67. Taghipour Y., Hajialyani M., Naseri R., et al. Nanoformulations of natural products for management of metabolic syndrome. *Int J Nanomedicine*. 2019; 14: 5303-5321.
68. Sabzichi M., Hamishehkar H., Ramezani F., et al. Luteolin-loaded phytosomes sensitize human breast carcinoma MDA-MB 231 cells to doxorubicin by suppressing Nrf2 mediated signalling. *Asian Pac J Cancer Prev*. 2014; 15(13): 5311-5316.
69. Deleanu M., Toma L., Sanda G., et al. Formulation of Phytosomes with Extracts of Ginger Rhizomes and Rosehips with Improved Bioavailability, Antioxidant and Anti-Inflammatory Effects In Vivo. *Pharmaceutics*. 2023; 15(4): 1066.
70. Nagpal N., Arora M., Swami G., Rageeb and Kapoor R. Designing of a phytosome dosage form with Tecomella undulata as a novel drug delivery for better utilization. *Pak J Pharm Sci*. 2016; 29(4): 1231-1235.
71. Hou Z., Li Y., uang Y., et al. Phytosomes loaded with mitomycin C-soybean phosphatidylcholine complex developed for drug delivery. *Mol Pharm*. 2013; 10(1): 90-101.
72. Ajazuddin. and Saraf S. Applications of novel drug delivery system for herbal formulations. *Fitoterapia*. 2010; 81(7): 680-689.
73. Kumar N., Goel R., Gaur P., et al. Development and evaluation of phytosome-loaded microsphere system for delivery of ginseng extract. *J Microencapsul*. 2021; 38(7-8): 496-506.
74. Sharma S. and Sahu A. Development, Characterization, and Evaluation of Hepatoprotective Effect of Abutilon indicum and Piper longum Phytosomes. *Pharmacognosy Res*. 2016; 8(1): 29-36.
75. Susilawati Y., Chaerunisa A. and Purwaningsih H. Phytosome drug delivery system for natural cosmeceutical compounds: Whitening agent and skin antioxidant agent. *Journal of advanced pharmaceutical technology & research*, 2021; *12*(4): 327–334.
76. Cotellese R., Ledda A., Belcaro G., et al. Anthocran Phytosome: Prevention of Recurring Urinary Infections and Symptoms after Catheterization. *J Diet Suppl*. 2023; 20(1): 55-67.
77. Magrone T., Pugliese V., Fontana S. and Jirillo E. Human use of Leucoselect Phytosome with special reference to inflammatory-allergic pathologies in frail elderly patients. *Curr Pharm Des*. 2014; 20(6): 1011-1019.
78. Rondanelli M., Perna S., Gasparri C., et al. Promising Effects of 3-Month Period of Quercetin Phytosome Supplementation in the Prevention of Symptomatic COVID-19 Disease in Healthcare Workers: A Pilot Study. *Life (Basel)*. 2022; 12(1): 66.
79. Marcheggiani F., Orlando P., Silvestri S., et al. CoQ_{10} Phytosomes Improve Cellular Ubiquinone Uptake in Skeletal Muscle Cells: An Ex Vivo Study Using CoQ_{10}-Enriched Low-Density Lipoproteins Obtained in a Randomized Crossover Study. *Antioxidants (Basel)*. 2023; 12(4): 964.
80. Telange D., Patil A., Pethe A., Fegade H., Anand S. and Dave V. Formulation and characterization of an apigenin-phospholipid phytosome (APLC) for improved solubility, in vivo bioavailability, and antioxidant potential. *Eur J Pharm Sci*. 2017; 108: 36-49.
81. Pastorelli D., Fabricio ASC., Giovanis P., et al. Phytosome complex of curcumin as complementary therapy of advanced pancreatic cancer improves safety and efficacy of gemcitabine: Results of a prospective phase II trial. *Pharmacol Res*. 2018; 132: 72-79.
82. Kidd P. Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts. *Altern Med Rev*. 2009; 14(3): 226-246.

83. Di Pierro F., Menghi A., Barreca A., Lucarelli M. and Calandrelli A. Greenselect Phytosome as an adjunct to a low-calorie diet for treatment of obesity: a clinical trial. *Altern Med Rev*. 2009; 14(2): 154-160.
84. Albalawi R., Binmahfouz L., Hareeri R., Shaik R. and Bagher A.M. Parthenolide Phytosomes Attenuated Gentamicin-Induced Nephrotoxicity in Rats via Activation of Sirt-1, Nrf2, OH-1, and NQO1 Axis. *Molecules*. 2023; 28(6): 2741.
85. El-Menshawe S., Ali A., Rabeh M., Khalil N.M. Nanosized soy phytosome-based thermogel as topical anti-obesity formulation: an approach for acceptable level of evidence of an effective novel herbal weight loss product. *Int J Nanomedicine*. 2018; 13: 307-318.
86. Direito R., Reis C., Roque L., et al. Phytosomes with Persimmon (*Diospyros kaki* L.) Extract: Preparation and Preliminary Demonstration of In Vivo Tolerability. *Pharmaceutics*. 2019; 11(6): 296.
87. Matias D., Rijo P. and Reis CP. Phytosomes as Biocompatible Carriers of Natural Drugs. *Curr Med Chem*. 2017; 24(6): 568-589.
88. Gnananath K., Sri Nataraj K. and Ganga Rao B. Phospholipid Complex Technique for Superior Bioavailability of Phytoconstituents. *Adv Pharm Bull*. 2017; 7(1): 35-42.
89. Hendawy O., Al-Sanea M., Elbargisy R., et al. Development of Olive Oil Containing Phytosomal Nanocomplex for Improving Skin Delivery of Quercetin: Formulation Design Optimization, In Vitro and Ex Vivo Appraisals. *Pharmaceutics*. 2023; 15(4): 1124.
90. Yu F., Li Y., Chen Q., et al. Monodisperse microparticles loaded with the self-assembled berberine-phospholipid complex-based phytosomes for improving oral bioavailability and enhancing hypoglycemic efficiency. *Eur J Pharm Biopharm*. 2016; 103: 136-148.
91. Zhang Z., Chen Y., Deng J., Jia X., Zhou J. and Lv H. Solid dispersion of berberine-phospholipid complex/TPGS 1000/SiO_2: preparation, characterization and in vivo studies. *Int J Pharm*. 2014; 465(1-2): 306-316.
92. Pooja D., Rupal J., G. S. C., et al. Phytosomes: A promising delivery system for anticancer agents by using phytochemicals in cancer therapy. *Cell Mol Biol (Noisy-le-grand)*. 2023; 69(14): 1-8.
93. Dundar N. A., Ozdemir S., Uzuner K., et al. Characterization of pomegranate peel extract loaded nanophytosomes and the enhancement of bio-accessibility and storage stability. *Food Chem*. 2023; 398: 133921.
94. Soltanzadeh M., Peighambardoust S., Ghanbarzadeh B., Mohammadi M. and Lorenzo JM. Chitosan Nanoparticles as a Promising Nanomaterial for Encapsulation of Pomegranate (*Punica granatum* L.) Peel Extract as a Natural Source of Antioxidants. *Nanomaterials (Basel)*. 2021; 11(6): 1439.
95. Human C., Aucamp M., de Beer D., van der Rijst M. and Joubert E. Food-grade phytosome vesicles for nanoencapsulation of labile *C*-glucosylated xanthones and dihydrochalcones present in a plant extract matrix-Effect of process conditions and stability assessment. *Food Sci Nutr*. 2023; 11(12): 8093-8111.
96. Tiwari R., Tiwari G., Sharma S. and Ramachandran V. An Exploration of Herbal Extracts Loaded Phyto-phospholipid Complexes (Phytosomes) Against Polycystic Ovarian Syndrome: Formulation Considerations. *Pharm Nanotechnol*. 2023; 11(1): 44-55.
97. Ortega-Pérez L., Ayala-Ruiz L., Magaña-Rodríguez O., et al. Development and Evaluation of Phytosomes Containing *Callistemon citrinus* Leaf Extract: A Preclinical Approach for the Treatment of Obesity in a Rodent Model. *Pharmaceutics*. 2023; 15(9): 2178.
98. Direito R., Reis C., Roque L., et al. Phytosomes with Persimmon (*Diospyros kaki* L.) Extract: Preparation and Preliminary Demonstration of In Vivo Tolerability. *Pharmaceutics*. 2019; 11(6): 296.
99. Abdelkader H., Longman M., Alany R. and Pierscionek B. Phytosome-hyaluronic acid systems for ocular delivery of L-carnosine. *Int J Nanomedicine*. 2016; 11: 2815-2827.

100. Jain P., Taleuzzaman M., Kala C., Kumar Gupta D., Ali A. and Aslam M. Quality by design (Qbd) assisted development of phytosomal gel of aloe vera extract for topical delivery. *J Liposome Res*.

101. Mi P. Stimuli-responsive nanocarriers for drug delivery, tumor imaging, therapy and theranostics. *Theranostics*. 2020; 10(10): 4557-4588.

102. Singh D., Rawat M., Semalty A. and Semalty M. Rutin-phospholipid complex: an innovative technique in novel drug delivery system- NDDS. *Curr Drug Deliv*. 2012; 9(3): 305-314.

103. Wang H., Cui Y., Fu Q., et al. A phospholipid complex to improve the oral bioavailability of flavonoids. *Drug Dev Ind Pharm*. 2015; 41(10): 1693-1703.

104. Sri K., Kondaiah A., Ratna J. and Annapurna A. Preparation and characterization of quercetin and rutin cyclodextrin inclusion complexes. *Drug Dev Ind Pharm*. 2007; 33(3): 245-253.

105. Saoji S., Raut N., Dhore P., Borkar C., Popielarczyk M. and Dave VS. Preparation and Evaluation of Phospholipid-Based Complex of Standardized Centella Extract (SCE) for the Enhanced Delivery of Phytoconstituents. *AAPS J*. 2016; 18(1): 102-114.

106. Telange D., Nirgulkar S., Umekar M., Patil A., Pethe A. and Bali N. Enhanced transdermal permeation and anti-inflammatory potential of phospholipids complex-loaded matrix film of umbelliferone: Formulation development, physico-chemical and functional characterization. *Eur J Pharm Sci*. 2019; 131: 23-38.

107. Freag M., Elnaggar Y. and Abdallah O. Lyophilized phytosomal nanocarriers as platforms for enhanced diosmin delivery: optimization and ex vivo permeation. *Int J Nanomedicine*. 2013; 8: 2385-2397.

108. Hanif H., Abdollahi V., Javani Jouni F., et al. Quercetin nano phytosome: as a novel anti-leishmania and anti-malarial natural product. *J Parasit Dis*. 2023; 47(2): 257-264.

109. Maramaldi G., Togni S., Pagin I., et al. Soothing and anti-itch effect of quercetin phytosome in human subjects: a single-blind study. *Clin Cosmet Investig Dermatol*. 2016; 9: 55-62.

110. Susilawati Y., Chaerunisa A., Purwaningsih H. Phytosome drug delivery system for natural cosmeceutical compounds: Whitening agent and skin antioxidant agent. *J Adv Pharm Technol Res*. 2021;12(4): 327-334.

111. Amit P., Tanwar Y., Rakesh S., Poojan P. Phytosome: Phytolipid drug delivery system for improving bioavailability of herbal drug. *J Pharm Sci Biosci Res.* 2013; 3: 51-7.

112. Patel J., Patel R., Khambholja K. and Patel N. An overview of phytosomes as an advanced herbal drug delivery system. *Asian J Pharm Sci.* 2009; 4: 363-71.

113. Aggarwal K. and Gupta A. and Chaturvedi S. Improvement in bioavailability of class III drug: Phytolipid delivery system. *Int J Pharm Pharm Sci.* 2012; 4(1): 38.

114. Mazumder A., Dwivedi A., du Preez J. and du Plessis J. In vitro wound healing and cytotoxic effects of sinigrin-phytosome complex. *Int J Pharm*. 2016; 498(1-2): 284.

115. Alholy T. and Khaddam, W. Extracellular Synthesis of Magnesium Oxide at Nano and Bulk Scale: Antifungal Effect Against Candida albicans, Aspergillus niger: Anti-fungal effect of MgO nano and bulk particles. *Jordan Journal of Pharmaceutical Sciences*. 2023; 16(4): 740–752.

116. Keerthi B., Pingali S. and Srinivas P. Formulation and evaluation of capsule of Ashwagandha phytosome. *Int J Pharm Sci Rev Res*. 2014; 29(2): 140.

117. Dhase S. and Saboo S. Preparation and evaluation of phytosome containing methanolic extract of leaves of Aegle marmelos (Bael). *Int J Pharm Technol Res*. 2015; 8(6): 232-3.

118. Surendra T., Dilip K., Lipika B. and Suresh K. A review on phytosomes, their characterization, advancement and potential for transdermal application. *J Drug Deliv Ther*. 2013; 3: 147-52.
119. Mishra B. Phytosomes: A promising system of herbal drug delivery. *J. Chem. Pharm. Res.* 2013; 2(4): 309-315.
120. Sikarwar M. A Review on Phytosome Technology as a Novel Approach to Improve the Bioavailability of Nutraceuticals. *Int J of Adv & tech, Planta Ind*. 2005; 1(2): 1-3.
121. Deshpande p., Pathak A. and Gothalwal R. Phytosomes: A promising system of herbal drug delivery. *J. New Bio. Rep.* 2014; 3(3): 212 – 220.
122. Bhattacharjee A. Phytosomes: A promising system of herbal drug delivery. *Int. J. Inno. Pharm. Sci. Res.* 2015; 3(6): 730-739.
123. Singha A., Saharanb V., Singha M. and Bhandaria A. Phytosome: Drug Delivery System for Polyphenolic Phytoconstituents. *Iran. J. Pharm. Sci*. 2011; 7(4): 209-219.
124. Rajendhiran N. and Bhattacharyya S. Preparation and Evaluation of Nanolipid Carriers of Bedaquiline- In vitro Evaluation and in silico Prediction. *Jordan Journal of Pharmaceutical Sciences*. 2024; 17(3): 450–467.

الفايتوزومات: منصة متطورة لتوصيل المواد الكيميائية النباتية من خلال تعزيز التوافر البيولوجي

سميتا كومبهار* [1]، فايشنافي جاغدال[2]، مانيش بهاتيا[3]

[1]قسم الكيمياء الصيدلانية، كلية سانجيفاني للتعليم والبحوث الصيدلانية مستقلة، كوبارجاون، ماهاراشترا، الهند.

[2]قسم الصيدلانيات، معهد نيرانت للصيدلة التابع لمنتدى أميبورفا، سولابور، الهند.

[3]قسم الكيمياء الصيدلانية، كلية بهاراتي فيديابيث للصيدلة، كولهابور، الهند.

ملخص

كلمة "phyto"تعني النبات، بينما كلمة "some"تعني الخلية .تتضمن هذه التقنية المبتكرة الحاصلة على براءة اختراع الجمع بين المستخلصات النباتية أو المكونات النباتية المحبة للماء مع الدهون الفوسفاتية لإنشاء مجمعات جزيئية مناسبة للدهون، مما يؤدي ليس فقط إلى تعزيز الامتصاص، ولكن أيضًا إلى التوافر البيولوجي .تم إجراء أبحاث واسعة النطاق من قبل العديد من العلماء لاستكشاف الطريقة عبر الجلد كوسيلة ممتازة لتوصيل المكونات النباتية .تحظى منتجات Phyto أو مستخلصات Phyto باهتمام كبير كمكملات غذائية في إدارة الالتهابات والسمية والسرطان وفقدان الوزن ومختلف الحالات التنكسية المزمنة .ومع ذلك، يتم إجراء تطورات ودراسات مستمرة في هذا المجال .كثيرًا ما تواجه هذه المنتجات مشكلات تتعلق بالاستقرار والتوافر البيولوجي .بمجرد استخراجها، تصبح المنتجات النباتية عرضة لعدم الاستقرار وقد لا تكون مناسبة للمرور عبر الغشاء البيولوجي .تعمل هذه التقنية على تعزيز محبة الماء للأدوية شديدة المحبة للدهون، وتصنيعها بشكل مناسب لتوصيل الدواء، وتعزيز محبة الدهون للمكونات النباتية بشكل مناسب لتسهيل التخلل عبر الغشاء الحيوي .لقد تم بالفعل إثبات استخدام الفايتوسومات لأغراض التجميل علميا .بالإضافة إلى ذلك، تقدم هذه المراجعة تحليلًا نسبيًا للجسيمات الشحمية والفايتوسومات، مع تسليط الضوء على التطورات الحالية في تكنولوجيا الفايتوسومات، ومعظمها في توصيل الأدوية عبر الجلد .يمكن أن يؤدي دمج مركبات البوليفينول في نظام توصيل قائم على الفوسفوليبيد مُجمَّع ذاتيًا، والمعروف باسم الفايتوسومات، إلى تحسين التوافر البيولوجي الضعيف عن طريق الفم بشكل كبير.

الكلمات الدالة: الفايتوسومات، مجمع الفسفوليبيد، التوافر البيولوجي، المكونات النباتية، الناقل النانوي.

* ***المؤلف المراسل: سميتا كومبهار***

smitakumbhar@gmail.com

تاريخ استلام البحث 2024/03/20 وتاريخ قبوله للنشر 2024/06/14.

Hydroethanolic Leaf Extract of *Murraya Koenigii*: Phytochemical Constituents and Biological Evaluation of its Toxicity and Antipyretic Activity in Wistar Albino Rats

Manisha Shrestha[1], Sindhu K C[1]*, Bipin Sah[1], Prabhat Kumar Jha[2], Sajan Khaitu[1] , Bipindra Pandey[2], Ram Kishor Yadav[2] , Ashish Gautam[1] , Binay Yadav[1]

[1]Chitwan Medical College, Tribhuvan University, Nepal
[2]Pokhara University, Pokhara, Nepal

ABSTRACT

Background: Fever, characterized by an elevated body temperature beyond the normal range, necessitates effective management. Traditional therapies rooted in indigenous knowledge prove effective, in addressing fever-related conditions for optimal well-being. This study explores the antipyretic potential of *Murraya koenigii*, a plant deeply rooted in traditional practices in Nepal.

Materials and Methods: The hydroethanol leaf extract of *Murraya koenigii* was subjected to phytochemical screening and acute toxicity assessment, followed by *I*n vivo antipyretic effects evaluated in male Wistar Albino rats using a yeast-induced fever model.

Results: Phytochemical analysis revealed the presence of bioactive compounds such as saponins, flavonoids, glycosides, phenols, tannins, and alkaloids. The acute toxicity study demonstrated the safety of *Murraya koenigii* extract up to 5000 mg/kg, highlighting its wide safety margin. *In vivo* antipyretics evaluation showed a significant ($p< 0.05$) temperature reduction at time 90 and 120 minutes by *Murraya koenigii* hydroethanolic extract (250mg/kg), comparable to the negative control group.

Conclusion: In conclusion, this study provides valuable insights into the phytochemical profile, safety, and antipyretics properties of *Murraya koenigii*, supporting its traditional use for fever management.

Keywords: *Murraya koenigii*, antipyretics, phytochemical, acute toxicity.

1. INTRODUCTION

Fever, a frequent increase in body temperature, is characterized by an elevated body temperature beyond the normal range (36.5-37.5°C) due to a raised hypothalamic set point [1]. The production of prostaglandins E2 in the preoptic area of the hypothalamus is necessary for altering neuronal firing rates, ultimately causing fever[2]. Antipyretic medication works by inhibiting cyclooxygenase-2 (COX-2) expression, which decreases the production of prostaglandin E2 (PGE2), the chief mediator of fever. Non-steroidal anti-inflammatory drugs (NSAIDs) like Diclofenac, aspirin and antipyretic drug are commonly used as antipyretic medication[3]. Due to the widespread adverse effects of synthetic medications, there has been a notable increase in interest in natural goods, leading to rise in studies aimed at examining their possible advantages[4,5]. The use of natural plant products as therapeutic solutions for fever and inflammation has become more well-known over the last 20 years due to continued study and the relatively low incidence of side effects when compared to manufactured drugs [6,7].

In Nepal, traditional methods for fever often involve the utilization of varieties medicinal plant species. One

***Corresponding author: Sindhu K.C.**
sindhukc119@gmail.com
Received: 05/04/2024 Accepted: 14/06/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2532

 © 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

such plant is *Murraya koenigii (M. koenigii)*, belonging to the Rutaceae family. Widely distributed in tropical and subtropical regions in Nepal. It has been traditionally employed in diverse cultural practices for its medicinal properties including hair care, fever control, digestive aid, diabetics management, stimulant, stomachic, analgesic, and therapeutic qualities that may be used to treat bug bites, diarrhea, and dysentery in addition to its antidepressant and anti-inflammatory qualities[8 9]. The fragrant leaves of Murraya koenigii are well-known for their usage as a culinary spice. Its powerful, characteristic perfume is mostly caused by four chemical compounds: O-phellandrene, p-caryophyllene, p-elemene, and p-gurjunene. Additionally, this plant has large concentrations of carbazole alkaloids, including isomurrayazoline, mahanine, koenine, koenigine, koenidine, and koenimbine[10].

Despite its extensive use, scientific investigations into the phytochemical constituents, acute toxicity profile, and antipyretic potency of *Murraya* species are very limited. Therefore, the study was designed to examine the antipyretic effects of *M. koenigii* leaf hydroethanolic extracts in rat models.The findings of this study aim to bridge the gap between traditional knowledge and scientific validation, providing valuable insights into the pharmacological properties of *M. koenigii* and its potential role in managing fever-related conditions.

2. MATERIALS AND METHODS

2.1 Plant sample collection, identification, and preparation

The leaves of *M. koenigii* were collected from the local place i.e., Bharatpur-2, Chitwan, Nepal in May 2016. The samples were identified and authenticated by the Agribotany Department of Agriculture and Forestry University of Rampur, Chitwan, Nepal, and were assigned with voucher specimen' number AG0223. The collected leaves were shade dried for 15 days and ground to coarse particles using a mechanical grinder.

2.2 Extraction

The powered leaves of *M. koenigii* were cold macerated for 24 hours in 70% ethanol in the ratio of 1:8 i.e., 8ml of ethanol was used for each g of *M. koenigii* powder. Then supernatant was filtered and separated from marc. The obtain *M. koenigii* extract was concentrated under rotatory evaporator. The concentrated slurry was then kept for five days in a vacuum desiccator to ensure complete dryness[11].

2.3 Phytochemical screening

The hydroethanolic extracts of *M. koenigii* leaves were assessed for alkaloids, saponins, tannins, flavonoids, glycosides, and phenolic compounds, using Mayers, Froath formation, Ferric chloride, Shinoda, Keller-Killiani and Lead acetate test method respectively [12 13 14].

2.4 Experimental animals

Male Wistar Albino rats (2-3 months old, average weight 150 g) were bought from the animal house department of plant resources, Kathmandu, Nepal, and were kept in polyethylene cages at 25 ± 2°C, 40–60% humidity, with a 12-hour light-dark cycle, and provided standard diet and water ad libitum housed at primate facility of the Chitwan medical college, Nepal. Ethical approval was obtained from Institution Research Committee (IRC) of Chitwan Medical College for the experiment with reference no. CMCIRC465.

2.5 Experimental design

2.5.1 Acute toxicity study

Male Wistar Albino rats were used to assess the toxicity of *Murraya koenigii* leaves hydroethanolic extract (MKLHE) following the guidelines outlined by OECD (Organization, for Economic Co-operation and Development) guideline 423. The rats were divided into four groups, each consisting of six rats and underwent a 12-hour fasting period while having access to water. The control group was given distilled water at dose 10 ml/kg whereas the other three groups received *M. koenigii* extract was dissolved in distilled water and administered at doses of 1000 mg/kg, 3000 mg/kg and 5000 mg/kg respectively.

Throughout 30 minutes, continuous monitoring was conducted to observe any changes in behavior, food and water intake as mortality. Subsequently, intermittent checks were performed from 4 to 24 hours after administration. These observations continued for 14 days[15].

2.5.2 Antipyretic study

Twenty-seven male wistar rats (140-150 g) were randomly divided into 3 groups and fasted overnight before the experiment with free access to water. The normal body temperature of each rat was measured rectally using a lubricated thermometer. After measuring the basal rectal temperature. A 15% baker's yeast suspension in 0.9% saline was prepared and injected subcutaneously to the each of rats. Eighteen hours after bakers's yeast injection, the animals were again restrained for rectal temperature recording, as described previously. Only rats that showed an increase in temperature of at least 1°C were used for this study. The MKHEE at the doses of 250 mg/kg were administered orally to one groups of animals. The negative control group received an 1ml of vehicle (0.9% saline), and the positive control group received paracetamol 150mg/kg) orally. Rectal temperature was measured at 30 minutes intervals for 2 hours after the extract/drug administration[16].

2.6 Data management and statistical analysis

The data are shown as mean ± standard deviation (n = 9). Data management and inferential analysis was done by SPSS version 16 software. *P values* ≤ *0.05* were regarded as a significant indicator.

3. RESULTS

3.1 Qualitative phytochemical analysis

Table 1, shows the phytochemical test results from *M. koenigii*. This table displays the presence of active phytochemical constituents, which are organic substances found in an extract.

Table 1. Phytochemical constituents present in M. koenigii

S.no	Test	Results
1.	Saponins	+
2.	Flavonoid	+
3.	Glycosides	+
4.	Phenols	+
5.	Tannins	+
6.	Alkaloids	+

+ = Presence**, -** = Absence

3.2Acute toxicity study

The acute toxicity study of *M. koenigii* extract in male Wistar Albino rats revealed no mortality or adverse reactions at doses up to 5000 mg/kg. Continuous monitoring for 30 minutes and subsequent observations over 14 days showed no signs of diarrhea, mortality, hair fall, or behavioral changes, indicating the safety of *M. koenigii* within the tested dose range.

3.3 In-vivo Antipyretic Effects

As shown in Table 2 over 120 minutes, MKLHE demonstrated a potential antipyretic effect in albino rats. At 90 and 120 minutes, MKLHE significantly ($p < 0.05$) reduced body temperature compared to the negative control (distilled water) and exhibited comparable efficacy to the standard (paracetamol), suggesting its promising role in fever management.

Table 2. In vivo antipyretic effect at different time intervals

Time (Minute)	Water for injection (0.9% saline) 1ml	Standard (Paracetamol) 150mg/kg	MKLHE 250mg/kg
0	101±0.4	100.83±0.50	100.94±0.45
30	101.23±0.39	100.1±0.45	101.3±0.3
60	101.2±0.33	99.16±0.25	99.33±0.25
90	100.73±0.2	98.33±0.15*	98.63±0.16*
120	100.4±0.13	97.96±0.11*	98.2±0.11*

Note: * indicates *P value ≤ 0.05* level of significance when compared with negative control

4. DISCUSSION

The qualitative phytochemical analysis of the hydro ethanol leaf extract of *M. koenigii* revealed the presence of various active constituents, including saponins, flavonoids, glycosides, phenols, tannins, and alkaloids (Table 1). A similar finding was reported by Yohanes et al, in their analysis of *Murray* species. These compounds are known for their diverse pharmacological activities, and their presence aligns with traditional knowledge and supports the exploration of *M. koenigii* for medicinal purposes[17]. Previous studies by Balakrishnan et. al have reported mahanine, mahanimbine, isomahanine, koenimbine, girinimbine, and isolongifolene as the principal chemical constituents present in Murraya species, showcasing pharmacological activity [18].

The acute toxicity study demonstrated the safety of *M. koenigii* extract in male Wistar Albino rats up to a dose of 5000 mg/kg. The absence of mortality or adverse reactions over a 14-day observation period suggests a wide safety margin. This finding is consistent with the finding of Menezes et al [19], who also observed no mortality or adverse reactions up to 5000mg/kg in their study on the safety profile of *M. panculata* .The wide safety margin observed in the present study reinforces the potential therapeutic use of the *M. koenigii* leaves.

The *in vivo* antipyretic effect of *Murray koenigii* leaves hydroethanolic extract was evaluated in Wistrar albino rats, indicating a reduction in body temperature at 90 and 120 minutes, comparable to the standard drug, paracetamol (150mg/kg) (Table 2). This finding supports the traditional use of *M. koenigii* for fever management and suggests its potential as a natural antipyretic agent. This data closely aligns with the work of Forkuo et al [20], who has reported a similar antipyretic effect with *M. koenigii* exotic a methanol leaf extract in their study. The comparison with 150 mg/kg paracetamol strengthens the credibility of the present finding, suggesting the potential of *M. koenigii* as a natural antipyretic agent. The presence of alkaloids, terpenoids and flavonoids in *M. koenigii*, as revealed in the phytochemical analysis, aligns with the observed antipyretic effect. Alkaloids, known for their ability to suppress cyclooxygenase (COX) enzymes, may contribute to the reduction of body temperature by modulation of inflammatory pathways, inhibiting of cyclooxygenase enzymes in the brain ,and effect on the central nervous system to regulate body temperature in a mechanism similar to the mechanism of Saction of paracetamol[21, 22]. The potential role of alkaloids in modulating fever might reinforce the traditional use of *M. koenigii* leaves for fever-related conditions[23]. As previous study carried out by Forkuo et al, on *Murraya exotica* (L.) found that at dose 300mg/kg the reduction in rectal temperature was 66.42% showcasing the antipyretic effect of *Murraya exotica* (L.) leaves extract[24].

5. CONCLUSION

Hence the comprehensive study highlights the phytochemical profile, safety, and antipyretic properties of *M. koenigii*. The results support its traditional use in fever management and suggest its potential as a valuable

therapeutic agent. This study also support the traditional use *M. koenigii* for fever management. Further investigation into the molecular mechanism of alkaloids obtained from *M. koenigii* and clinical applications of *M. koenigii* is necessary to justify its medicinal potential.

Acknowledgement

We extend our gratitude to Chitwan Medical College for generously providing laboratory facilities essential for conducting our experiment and the local informants in Chitwan, Nepal, whose priceless sharing of traditional plant knowledge was essential to our research.

Funding for the study

No funding was provided to carry out these experiments.

Conflict of Interest

The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

REFERENCES

1. Del Bene, V. E. Temperature. In *Clinical Methods: The History, Physical, and Laboratory Examinations*; Walker, H. K., Hall, W. D., Hurst, J. W., Eds.; Butterworths: Boston, 1990.
2. Scammell, T. E.; Elmquist, J. K.; Griffin, J. D.; Saper, C. B. Ventromedial Preoptic Prostaglandin E2 Activates Fever-Producing Autonomic Pathways. *The Journal of Neuroscience* 1996, *16* (19), 6246. https://doi.org/10.1523/JNEUROSCI.16-19-06246.1996.
3. Lee, J. J.; Simmons, D. L. Antipyretic Therapy: Clinical Pharmacology. *Handb Clin Neurol* 2018, *157*, 869–881. https://doi.org/10.1016/B978-0-444-64074-1.00054-9.
4. Ugoeze, K. C.; Aja, P. C.; Nwachukwu, N.; Chinko, B. C.; Egwurugwu, J. N. Assessment of the Phytoconstituents and Optimal Applicable Concentration of Aqueous Extract of Azadirachta Indica Leaves for Wound Healing in Male Wistar Rats. *Thai Journal of Pharmaceutical Sciences (TJPS)* 2021, *45* (1).
5. Naitik, P.; Prakash, T.; Kotresha, D.; Rao, N. R. Effect of Terminalia Catappa on Lipid Profile in Transplanted Fibrosarcoma in Rats. *Indian J Pharmacol* 2012, *44* (3), 390–392. https://doi.org/10.4103/0253-7613.96345.
6. Chaachouay, N.; Zidane, L. Plant-Derived Natural Products: A Source for Drug Discovery and Development. *Drugs and Drug Candidates* 2024, *3* (1), 184–207. https://doi.org/10.3390/ddc3010011.
7. Alkhatib, R. Chemical Composition of Essential Oils Total Phenols and Antioxidant Activity of Achillea Fragrantissima and A. Santolina Grown in Syria. *Jordan Journal of Pharmaceutical Sciences* 2024, *17* (3), 594–602. https://doi.org/10.35516/jjps.v17i3.2389.
8. Kunwar, R. M.; Shrestha, K. P.; Bussmann, R. W. Traditional Herbal Medicine in Far-West Nepal: A Pharmacological Appraisal. *J Ethnobiol Ethnomed* 2010, *6*, 35. https://doi.org/10.1186/1746-4269-6-35.
9. Rahman, M. M.; Gray, A. I. A Benzoisofuranone Derivative and Carbazole Alkaloids from Murraya Koenigii and Their Antimicrobial Activity. *Phytochemistry* 2005, *66* (13), 1601–1606. https://doi.org/10.1016/j.phytochem.2005.05.001.
10. Franyoto, Y. D.; Nurrochmad, A.; Fakhrudin, N. Murraya Koenigii L. Spreng.: An Updated Review of Chemical Composition, Pharmacological Effects, and Toxicity Studies. *J App Pharm Sci* 2024. https://doi.org/10.7324/JAPS.2024.169254.
11. Bitwell, C.; Indra, S. S.; Luke, C.; Kakoma, M. K. A Review of Modern and Conventional Extraction Techniques and Their Applications for Extracting Phytochemicals from Plants. *Scientific African* 2023, *19*, e01585. https://doi.org/10.1016/j.sciaf.2023.e01585.

12. Larbie, C.; Owusu Nyarkoh, C.; Owusu Adjei, C. Phytochemical and Safety Evaluation of Hydroethanolic Leaf Extract of Tecoma Stans (L.) Juss. Ex Kunth. *Evid Based Complement Alternat Med* 2019, *2019*, 7417624. https://doi.org/10.1155/2019/7417624.
13. Bose, S.; Mandal, S. K.; Das, P.; Nandy, S.; Das, A.; Dutta, D.; Chakraborti, C.; Sarkar, D.; Dey, S. Comparative Evaluation of Anti-Inflammatory, Antipyretic and Analgesic Properties of Ixora Coccinea and Mussaenda Frondosa (Rubiaceae) Leaves. *Jordan Journal of Pharmaceutical Sciences* 2020, *13* (3).
14. Hossain, M. E.; Aziz, M. A.; Vabna, N. J.; Akter, M. I.; Hossain, S.; Sarker, S.; Mazumder, K. Phytochemical Screening and Pharmacological Evaluation of the Methanolic Extract of Cissus Elongata Roxb. Leaves. *Jordan Journal of Pharmaceutical Sciences* 2022, *15* (4), 449–460. https://doi.org/10.35516/jjps.v15i4.670.
15. Malik, M. K.; Bhatt, P.; Singh, J.; Kaushik, R. D.; Sharma, G.; Kumar, V. Preclinical Safety Assessment of Chemically Cross-Linked Modified Mandua Starch: Acute and Sub-Acute Oral Toxicity Studies in Swiss Albino Mice. *ACS Omega* 2022, *7* (40), 35506–35514. https://doi.org/10.1021/acsomega.2c01309.
16. Naiemur Rahman, M.; Shahin Ahmed, K.; Ahmed, S.; Hossain, H.; Shahid Ud Daula, A. Integrating in Vivo and in Silico Approaches to Investigate the Potential of Zingiber Roseum Rhizome Extract against Pyrexia, Inflammation and Pain. *Saudi J Biol Sci* 2023, *30* (4), 103624. https://doi.org/10.1016/j.sjbs.2023.103624.
17. Abeysinghe, D. T.; Kumara, K. A. H.; Kaushalya, K. A. D.; Chandrika, U. G.; Alwis, D. D. D. H. Phytochemical Screening, Total Polyphenol, Flavonoid Content, in Vitro Antioxidant and Antibacterial Activities of Sri Lankan Varieties of Murraya Koenigii and Micromelum Minutum Leaves. *Heliyon* 2021, *7* (7), e07449. https://doi.org/10.1016/j.heliyon.2021.e07449.
18. Balakrishnan, R.; Vijayraja, D.; Jo, S.-H.; Ganesan, P.; Su-Kim, I.; Choi, D.-K. Medicinal Profile, Phytochemistry, and Pharmacological Activities of Murraya Koenigii and Its Primary Bioactive Compounds. *Antioxidants (Basel)* 2020, *9* (2), 101. https://doi.org/10.3390/antiox9020101.
19. Menezes, I. R. A.; Santana, T. I.; Varela, V. J. C.; Saraiva, R. A.; Matias, E. F. F.; Boligon, A. A.; Athayde, M. L.; Coutinho, H. D. M.; Costa, J. G. M.; Rocha, J. B. T. Chemical Composition and Evaluation of Acute Toxicological, Antimicrobial and Modulatory Resistance of the Extract of Murraya Paniculata. *Pharm Biol* 2015, *53* (2), 185–191. https://doi.org/10.3109/13880209.2014.913068.
20. Forkuo, A. D.; Mensah, K. B.; Ameyaw, E. O.; Antwi, A. O.; Kusi-Boadum, N. K.; Ansah, C. Antiplasmodial and Antipyretic Activity and Safety Evaluation of the Methanolic Leaf Extract of Murraya Exotica (L.). *J Parasitol Res* 2020, *2020*, 1308541. https://doi.org/10.1155/2020/1308541.
21. Ain, Q. U.; Iqbal, M. O.; Khan, I. A.; Bano, N.; Naeem, M.; Jamaludin, M. I.; Devaraj, S. Phytochemical, Antioxidant, Antipyretic and Anti-Inflammatory Activities of Aqueous-Methanolic Leaf Extract of Mangifera Indica. *Am J Transl Res* 2023, *15* (7), 4533–4543.
22. Panche, A. N.; Diwan, A. D.; Chandra, S. R. Flavonoids: An Overview. *J Nutr Sci* 2016, *5*, e47. https://doi.org/10.1017/jns.2016.41.
23. Ahmad, I.; Khan, H.; Gilani, A.-U.-H.; Kamal, M. A. Potential of Plant Alkaloids as Antipyretic Drugs of Future. *Curr Drug Metab* 2017, *18* (2), 138–144. https://doi.org/10.2174/1389200218666170116102625.
24. Forkuo, A. D.; Mensah, K. B.; Ameyaw, E. O.; Antwi, A. O.; Kusi-Boadum, N. K.; Ansah, C. Antiplasmodial and Antipyretic Activity and Safety Evaluation of the Methanolic Leaf Extract of *Murraya Exotica (L.). Journal of Parasitology Research* 2020, *2020*, e1308541. https://doi.org/10.1155/2020/1308541.

مستخلص الأوراق الهيدروإيثنولية من *Murraya koenigii*: المكونات الكيميائية النباتية والتقييم البيولوجي لسميتها ونشاطها الخافض للحرارة في فئران ويستار ألبينو

مانيشا شريستا[1*]، سيندهو ك. س.[1]، بيبين ساه[1]، بربهات كومار جا[2]، ساجان خيتو[1]، بيبندرا باندي[2]، رام كيشور يادا ف[2]، أشيش غوتام[1]، بيناي يادا ف[1]، بوجا بوديل[1]

[1]كلية شيتوان الطبية، جامعة تريبهوفان، نيبال

[2]جامعة بوخارا، بوخارا، نيبال

ملخص

الخلفية: الحمى، التي تتميز بارتفاع درجة حرارة الجسم إلى ما هو أبعد من المعدل الطبيعي، تتطلب إدارة فعالة .تثبت العلاجات التقليدية المتجذرة في معارف السكان الأصليين فعاليتها، حيث تعالج الظروف المرتبطة بالحمى لتحقيق الرفاهية المثلى. تستكشف هذه الدراسة إمكانات خافض للحرارة لنبات Murraya koenigii، وهو نبات متجذر بعمق في الممارسات التقليدية في نيبال .

المواد والطرق: تم إخضاع مستخلص أوراق الهيدروإيثانول من Murraya koenigii للفحص الكيميائي النباتي وتقييم السمية الحادة متبوعًا بتأثيرات خافضة للحرارة في الجسم الحي تم تقييمها في ذكور فئران ويستار ألبينو باستخدام نموذج الحمى الناجم عن الخميرة.

النتائج: كشف التحليل الكيميائي النباتي عن وجود مركبات نشطة بيولوجيا مثل الصابونين والفلافونويدات والجليكوسيدات والفينولات والعفص والقلويدات .أظهرت دراسة السمية الحادة سلامة مستخلص موراي كونيجي حتى 5000ملغم/كغم، مما يسلط الضوء على هامش الأمان الواسع .أظهر تقييم خافضات الحرارة في الجسم الحي انخفاضًا كبيرًا في درجة الحرارة ($p< 0.05$) في الوقت 90و 120دقيقة بواسطة مستخلص Murraya koenigii الهيدروإيثانوليك (250)مجم/كجم، مقارنة بمجموعة التحكم السلبية.

الخلاصة: في الختام، توفر هذه الدراسة رؤى قيمة حول خصائص المواد الكيميائية النباتية والسلامة وخافضات الحرارة في Murraya koenigii، مما يدعم استخدامها التقليدي لإدارة الحمى.

الكلمات الدالة: موراي كونيجي، خافضات الحرارة، كيميائية نباتية، سمية حادة.

[*] ***المؤلف المراسل: سيندهو ك. س.***

sindhukc119@gmail.com

تاريخ استلام البحث 2024/04/05 وتاريخ قبوله للنشر 2024/06/14.

INutrition Support Pharmacy Services in Critically Ill Cancer Patients Admitted to Intensive Care Units: A Retrospective Analysis of Clinical Pharmacists' Interventions

Nadine N. Abdelhadi[1*], Alaa Dabbous[2], Saad Jaddoua[2]

[1] Faculty of Pharmacy, Aqaba University of Technology, Aqaba, Jordan.
[2] Department of Pharmacy, King Hussein Cancer Centre, Amman, Jordan.

ABSTRACT

Objectives: This study aimed to analyze clinical pharmacists' interventions in managing nutrition support therapy in critically ill cancer patients admitted to intensive care units.

Methods: A retrospective analysis of 9949 electronically reported clinical pharmacist interventions of patients admitted to ICU from January 2020 to December 2022 was conducted. All patients' records with clinical pharmacists' interventions related to nutrition support therapy in ICU cancer patients were included and analyzed.

Results: The number of interventions for managing nutrition support therapy was 95 (0.95 %). Parenteral nutrition (n = 83, 87.4 %) was the most frequently used class of nutrition support therapies. The evaluation, adjustment, and monitoring of total parenteral nutrition (n = 75, 78.9 %) was the most frequent intervention of clinical pharmacists. The acceptance rate of clinical pharmacists' interventions in nutrition support therapy by physicians was 100 %.

Conclusion: Clinical pharmacists have a role in managing specialized nutrition support therapy in critically ill cancer patients. The prevailing clinical pharmacists' intervention was evaluating, adjusting, and monitoring total parenteral nutrition. More studies are needed to investigate the barriers that prevent the application of nutrition support pharmacy services in Jordan and to find the impact of these services on patient outcomes.

Keywords: Clinical pharmacy services, nutrition support pharmacy, cancer, critical care.

1. INTRODUCTION

According to the American College of Clinical Pharmacy (ACCP), clinical pharmacy is the area of pharmacy concerned with the science and practice of rational medicine use [1]. Clinical pharmacy practice is based on the philosophy of pharmaceutical care, which aims to enhance patient care and obtain optimal patient outcomes [1].

Clinical pharmacists are healthcare professionals who specialize in providing direct patient care. The clinical pharmacy discipline has evolved to include many specialties [1]. The Board of Pharmacy Specialties (BPS) administers pharmacist certifications in various specialties ,including nutrition support pharmacy, critical care pharmacy, emergency medicine pharmacy, geriatric pharmacy, oncology pharmacy, paediatric pharmacy, and pharmacotherapy [2]. Nutrition Support Pharmacy is a speciality that provides optimum care to patients receiving specialized nutrition support, including parenteral or enteral nutrition, by qualified pharmacists [2].

Nutrition therapy includes oral, enteral, and parenteral nutrition to maintain optimal health [3]. Nutrition Support Therapy provides nutrients enterally or parenterally to prevent or treat malnutrition. Enteral nutrition (EN), or "tube feeding," is a type of nutrition support therapy in

[*]***Corresponding author: Nadine N. Abdelhadi***
nadine_abdelhadi@yahoo.com
Received: 09/04/2024 Accepted: 14/06/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.2540

 © 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

which patients can receive their nutritional needs by administering liquid formulas via enteral access devices or feeding tubes that directly access the stomach or small intestine [3,4]. Parenteral Nutrition is another type of nutrition support therapy in which patients meet their nutritional needs by administering a sterile intravenous (IV) solution containing carbohydrates, lipids, protein, vitamins, minerals, fluid, and other nutrients. Parenteral nutrition is used when a patient's nutritional needs could not be met through the gastrointestinal GI tract alone. [3,4].

Nutrition support professionals (NSPs) can be pharmacists, dietitians, nurses, physicians, or other healthcare providers [4]. Nutrition support pharmacists compound the prescribed parenteral nutrition formulation. They also provide nutrition support pharmacy services to patients [16]. Nutrition support clinical pharmacists may work independently or as part of the healthcare team [17,18]. Clinical pharmacists are essential members of critical care health teams and contribute to the safety and management of medications for this vulnerable population [16-25]. They provide various services, including medication reconciliation, drug monitoring, and drug information services, which lead to minimizing drug-related errors and optimizing medication therapy in intensive care units (ICU) [16-20]. Clinical pharmacists also provide nutrition support pharmacy services. These activities improve health quality, reduce mortality, improve patient outcomes, and decrease healthcare-related costs [16-20].

According to recent guidelines and recommendations, timely nutritional therapy should be carefully considered for malnourished or at-risk malnutrition patients undergoing anticancer management [20,21]. When oral intake is inadequate, enteral or parenteral nutrition should be considered if enteral nutrition is not adequate or feasible. The timing of nutritional interventions is critical, as early intervention may improve cancer patients' perceived quality of life. Nutrition therapy targeting the management of caloric deficit has improved patient outcomes [20-25].

The current healthcare system's focus on providing high-quality, affordable care imposes many challenges on health workers [5,6]. Preventing and managing malnutrition during hospitalization optimizes the quality of care, improves clinical outcomes, and reduces costs [6,7,8]. Although studies' findings are contrasting, available evidence showed that early nutrition support can reduce complications, duration of hospital stay, hospital readmission rates, mortality, morbidity, and costs. Optimized Nutrition support therapy is expected to save costs and enhance clinical outcomes in hospitalized patients [5-15].

A limited number of studies explored the role of clinical pharmacists in the Middle East [31-35]. Few studies investigated the role of clinical pharmacists in Jordan. However, none of these studies investigated the role of clinical pharmacists in specialized nutrition support therapy [36,37,38]. King Hussein Cancer Centre (KHCC) is a specialized cancer centre in Jordan. Clinical pharmacy services at KHCC cover all inpatient services and some outpatient clinics. Clinical pharmacists review all patients' medication orders, participate in clinical discussions with physicians and other healthcare professionals on a daily basis, and provide comprehensive therapeutic care plans [39].

Based on the above and due to the lack of information about the role of nutrition support pharmacy services in critically ill cancer patients in Jordan, the aim of this study was to analyze the interventions of clinical pharmacists in managing nutrition support therapy in ICU cancer patients at KHCC.

2. METHODS

The study protocol was approved by the Institutional Review Board (IRB) at King Hussein Cancer Centre on 25 Oct 2021 with approval number RC/2021/153.

A retrospective analysis of electronically reported

clinical pharmacist interventions in 9949 patients admitted to intensive care units at KHCC in Amman, Jordan, from January 2020 to December 2022 was conducted. All patient records with clinical pharmacist interventions for managing nutrition support pharmacy services in ICU cancer patients at King Hussein Cancer Centre were included.

Nutrition support pharmacy (NSP) services were classified into enteral nutrition (EN) and parenteral nutrition (PN). Reported clinical pharmacists' interventions for managing nutrition support pharmacy (NSP) services were classified into the following categories: Lab Evaluation, order clarification, recommendation/initiation,
Recommendation/discontinuation, and total parenteral nutrition (TPN) evaluation/adjustment/monitoring. The time clinical pharmacists took to intervene was reported and recorded by clinical pharmacists in the pharmacy database. Times were collected and analyzed for all interventions. Patients' age groups were classified into two categories: the adult group for patients older than 18 and the paediatric group for patients 18 or under. Descriptive statistics were utilized to evaluate the results in frequencies and percentages.

Mann-Whitney U test was used to compare the means of the time clinical pharmacists took to intervene in the paediatric and adult ICU services groups and between the enteral and parenteral nutrition groups.

Analysis was performed using the Jamovi statistical package 2022 [29,30]. A p-value less than 0.05 was considered significant.

3. RESULTS

The number of interventions related to managing nutrition support therapy was 95 (0.95 %). The acceptance rate of clinical pharmacists' interventions in nutrition support therapy by physicians at KHCC was 100 %.

Most of the study population were males ($n = 61$, 64.2%). The average age of females was 40.9 years (SD ± 25.6), and the average age of males was 36.6 years (SD ± 25.4). The study population comprised adult patients ($n = 56$, 58.9 %) and paediatric patients ($n = 39$, 41.1%). The descriptives of age are described in Table 1.

Table 1: Participants age

	Age (years)
N	95
Missing	5
Median	44
Standard deviation	25.4
IQR	47.5
Minimum	2
Maximum	83
25th percentile	13.0
50th percentile	44.0
75th percentile	60.5

3.1. Distribution of participants based on admission date:

The majority of participants were admitted to ICU in the year 2022. The number of patients admitted to the ICU in 2022 was ($n = 43$, 45.3%). The number of patients admitted in 2021 was ($n = 28$, 29.5 %), and in 2020 was ($n = 24$, 25.3).

3.2. Analysis of nutrition support therapies in ICU cancer patients:

Parenteral nutrition ($n = 83$, 87.4 %) was found to be the most used class of nutrition support therapies in ICU cancer patients. The frequency of nutrition support pharmacy services according to age and gender is described in Table 2.

Table 2: Frequencies of nutrition support therapies

Nutrition support therapies	Age group	Gender	Counts	% of Total	Cumulative %
Enteral nutrition	Adult	F	10	10.5 %	10.5 %
		M	2	2.1 %	12.6 %
	Pediatric	F	0	0.0 %	12.6 %
		M	0	0.0 %	12.6 %
Parenteral nutrition	Adult	F	12	12.6 %	25.3 %
		M	32	33.7 %	58.9 %
	Pediatric	F	12	12.6 %	71.6 %
		M	27	28.4 %	100.0 %

3.3. Analysis of clinical pharmacists' interventions in nutrition support pharmacy services

The evaluation, adjustment, and monitoring of TPN ($n = 75$, 78.9 %) was found to be the most frequent intervention of clinical pharmacists in the management of nutrition support therapy in ICU cancer patients, followed by discontinuation of the nutrition support therapy ($n = 14$, 14.7 %). Table 2 depicts the frequencies of drug interventions.

Table 2: Frequencies of Intervention

Intervention	Counts	% of Total	Cumulative %
Lab Evaluation	1	1.1 %	1.1 %
Order clarification	3	3.2 %	4.2 %
Recommendation/ Discontinuation	14	14.7 %	18.9 %
Recommendation/initiation	2	2.1 %	21.1 %
TPN evaluation/adjustment/monitoring	75	78.9 %	100.0 %

3.4. Analysis of time taken by clinical pharmacists to intervene:

The number of interventions related to managing nutrition support therapy was 95 (0.95 %). The sum of times taken was 1824 minutes. The minimum time was 2, and the maximum time was 45 minutes. Tables 3 and 4 describe the distribution of intervention time taken based on age, gender, and type of intervention.

Table 3: Distribution of time taken by clinical pharmacists (according to gender and age group)

	Age group	Gender	Time Taken
N	Adult	F	22
		M	34
	Pediatric	F	12
		M	27
Missing	Adult	F	0
		M	0
	Pediatric	F	0
		M	0
Median	Adult	F	15.0
		M	20.0
	Pediatric	F	20.0
		M	20

	Age group	Gender	Time Taken
Standard deviation	Adult	F	4.93
		M	8.50
	Pediatric	F	7.72
		M	8.13
IQR	Adult	F	7.50
		M	0.00
	Pediatric	F	0.00
		M	0.00
Minimum	Adult	F	2
		M	2
	Pediatric	F	15
		M	15
Maximum	Adult	F	20
		M	45
	Pediatric	F	45
		M	45
25th percentile	Adult	F	11.3
		M	20.0
	Pediatric	F	20.0
		M	20.0
50th percentile	Adult	F	15.0
		M	20.0
	Pediatric	F	20.0
		M	20.0
75th percentile	Adult	F	18.8
		M	20.0
	Pediatric	F	20.0
		M	20.0

Table 4: Distribution of time taken by clinical pharmacists (according to type of intervention)

	Intervention	Time Taken
N	Lab Evaluation	1
	Order clarification	3
	Recommendation/ Discontinuation	14
	Recommendation/initiation	2
	TPN evaluation/adjustment/monitoring	75
Missing	Lab Evaluation	0
	Order clarification	0
	Recommendation/ Discontinuation	0
	Recommendation/initiation	0
	TPN evaluation/adjustment/monitoring	0
Median	Lab Evaluation	5
	Order clarification	10
	Recommendation/ Discontinuation	15.0
	Recommendation/initiation	15.0

	Intervention	Time Taken
	TPN evaluation/adjustment/monitoring	20
Standard deviation	Lab Evaluation	NaN
	Order clarification	0.00
	Recommendation/ Discontinuation	4.73
	Recommendation/initiation	0.00
	TPN evaluation/adjustment/monitoring	7.78
IQR	Lab Evaluation	0.00
	Order clarification	0.00
	Recommendation/ Discontinuation	0.00
	Recommendation/initiation	0.00
	TPN evaluation/adjustment/monitoring	0.00
Minimum	Lab Evaluation	5
	Order clarification	10
	Recommendation/ Discontinuation	2
	Recommendation/initiation	15
	TPN evaluation/adjustment/monitoring	2
Maximum	Lab Evaluation	5
	Order clarification	10
	Recommendation/ Discontinuation	15
	Recommendation/initiation	15
	TPN evaluation/adjustment/monitoring	45
25th percentile	Lab Evaluation	5.00
	Order clarification	10.0
	Recommendation/ Discontinuation	15.0
	Recommendation/initiation	15.0
	TPN evaluation/adjustment/monitoring	20.0
50th percentile	Lab Evaluation	5.00
	Order clarification	10.0
	Recommendation/ Discontinuation	15.0
	Recommendation/initiation	15.0
	TPN evaluation/adjustment/monitoring	20.0
75th percentile	Lab Evaluation	5.00
	Order clarification	10.0
	Recommendation/ Discontinuation	15.0
	Recommendation/initiation	15.0
	TPN evaluation/adjustment/monitoring	20.0

3.6. Time taken by clinical pharmacist to intervene: enteral nutrition compared to PN

Mann-Whitney U test was conducted to compare the time clinical pharmacists took to intervene in enteral nutrition compared to PN. The test showed a difference between population means ($p<0.001$, 95% Confidence Interval). The results are shown in table 5.

Table 5: Mann Whitney U Test

						95% Confidence Interval		
		Statistic	p	Mean difference	Lower	Upper		Effect Size
Time Taken	Mann-Whitney U	121	< .001	-5.00	-10.00	-5.00	Rank biserial correlation	0.757

Note. H_a $\mu_{\text{Enteral feeding}} \neq \mu_{\text{TPN}}$

3.7. Time took by clinical pharmacists to intervene: paediatric compared to adult

Mann Whitney U Test was conducted to compare the time clinical pharmacists took to intervene in a paediatric group compared to the adult group. The test showed a difference between population means ($p<0.001$, **95% Confidence Interval**). The results are shown in table 6.

Table 6: Mann Whitney U Test

						95% Confidence Interval		
		Statistic	**p**	**Mean difference**	**Lower**	**Upper**		**Effect Size**
Time Taken	Mann-Whitney U	706	0.001	-1.88e−5	-5.00	-5.60e−5	Rank biserial correlation	0.354

Note. H_a $\mu_{\text{Adult}} \neq \mu_{\text{Pediatric}}$

4. DISCUSSION:

Few studies have investigated the role of clinical pharmacists in intensive care units in general [20-28]. This study was conducted at KHCC, a specialized cancer centre in Jordan. KHCC is considered a role model for other hospitals in Jordan as it is the only centre providing specialized nutrition support pharmacy services.

Despite the low number of clinical pharmacists' interventions in nutrition support therapy (0.95%), it is considered significant as it is the first experience in Jordan. More studies are needed to investigate the barriers that prevent applying nutrition support pharmacy services in Jordan. Further studies are also required to determine the impact of applying these services on patient outcomes.

This study found that clinical pharmacists had provided direct patient care to patients receiving nutrition support therapy, contrasting the findings of a study investigating the pharmacist's role in parenteral nutrition therapy in Kuwait [32]. The Kuwaiti study found that pharmacists in six governmental hospitals and one private hospital in Kuwait mainly performed technical tasks such as compounding total parenteral nutrition with minimal role in providing direct patient care [32].

This is the first study to describe clinical pharmacists' interventions in managing nutrition support therapy in the ICU cancer setting up to the authors' knowledge. This is one of the strengths of the current study. Our research team found that the most frequent interventions of clinical pharmacists were related to parenteral nutrition, followed by enteral nutrition. These findings were consistent with a prospective Chinese study conducted over one year in an ICU at an academic hospital in China [32]. Our research team also found that the most frequent interventions of clinical pharmacists in the management of nutrition support therapy in ICU cancer patients were the evaluation, adjustment, and monitoring of TPN ($n = 75$, 78.9 %) followed by discontinuation of the nutrition support therapy ($n = 14$, 14.7 %). This differs from the findings of the aforementioned Chinese study, which found that parenteral prescription and delivery are the most frequent interventions [31]. This difference might be explained by the fact that the involvement of clinical pharmacists in the management of nutrition support therapy practice is relatively new, and this is the first experience in Jordan. Another study in the literature found that the top intervention of nutrition support service

pharmacists was general laboratory monitoring, which is consistent with the current study[33].

This study found that the number of clinical pharmacists' interventions related to nutrition support therapy was relatively low compared to the total number of clinical pharmacists' interventions in the ICU. This finding is consistent with a survey of American nutrition support clinicians, in which 23% stated that their institutions did not have dedicated pharmacists to review parenteral nutrition orders [35]. This highlights the need for more active participation of clinical pharmacists in managing nutrition support therapy.

This was the first study to investigate the mean time the clinical pharmacist took to intervene in the management of nutrition support therapy in the cancer ICU setting. To the best of our knowledge, no previous studies are in the literature, which is another strength of this study.

The interventions in this study took between 2 and 45 minutes, while the findings of a related study showed that most pharmacist interventions in a university hospital setting took between 15 and 30 minutes to complete [35]. The current study was conducted in the ICU at a specialized cancer centre, while the other study in the literature was conducted at a university hospital in different departments. That would explain the difference between the two studies' findings. More studies are needed to investigate the factors affecting the time needed to provide clinical pharmacy interventions across different populations and health services. It would benefit the strategic planning and management of clinical pharmacy services.

The findings of this study highlighted the importance of exploring the time clinical pharmacists take to make interventions in the management of nutrition support therapy in ICU settings. These findings pave the way for future studies in different settings to determine the efficiency of clinical pharmacy services. More studies are needed to investigate the barriers that prevent applying nutrition support pharmacy services in Jordan. Further studies are also required to determine the impact of applying this service on patient outcomes.

The current study's limitation is the likely less-than-perfect documentation of intervention due to the retrospective design, which should be acknowledged. In addition, all interventions were included without running a quality appraisal for their content.

5. Conclusion:

This retrospective analysis described clinical pharmacist-delivered interventions in managing nutrition support therapy in the ICU cancer setting in Jordan. Clinical pharmacists have a role in managing specialized nutrition support therapy. The prevailing clinical pharmacist intervention was TPN's evaluation, adjustment, and monitoring. Nutrition support pharmacy services for critically ill cancer patients are uncommon in Jordan and could only be reported at KHCC. The number of clinical pharmacists' interventions related to nutrition support therapy was relatively low compared to the total number of clinical pharmacists' interventions in the ICU. However, this is considered significant as it is the first experience in Jordan. More studies are needed to investigate the barriers that prevent applying nutrition support pharmacy services in Jordan. Further studies are required to determine the impact of applying this service on patients, the cost of treatment, and the length of hospitalization.

Funding

No specific funding was obtained for this work.

Conflict of interest

No potential conflict of interest relevant to this study was reported.

REFERENCES

1. Definition of Clinical Pharmacy ACCP. Available at: https://www.accp.com/stunet/compass/definition.aspx (Accessed: 01 March 2024).
2. BPS specialties, Board of Pharmacy Specialties. 2024. Available at: https://bpsweb.org/bps-specialties/
3. ASPEN, What Is Nutrition Support Therapy? Nutritioncare.org. 2024. Available from: https://www.nutritioncare.org/About_Clinical_Nutrition/What_is_Nutrition_Support_
4. ASPEN | What Is a Nutrition Support Professional [Internet]. Nutritioncare.org. 2024. Available from: https://www.nutritioncare.org/what-is-a-NSP/
5. McClave S., DiBaise J., Mullin G. and Martindale R. ACG Clinical Guideline: Nutrition Therapy in the Adult Hospitalized Patient. *American Journal of Gastroenterology*. 2016; 111(3):315-334.
6. American Society Health-System Pharmacists. ASHP guidelines on the safe use of automated compounding devices for the preparation of parenteral nutrition admixtures. *Am J Health-Syst Pharm*. 2000; 57:1343—8
7. ASPEN Board of Directors and the clinical guidelines task force. Guidelines for the use of parenteral and enteral nutrition in adult and paediatric patients. *J Parenter Enteral Nutr*. 2002; 26:1SA—138SA
8. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient JPEN. 2016; 2(40):159 – 211.
9. ASPEN Clinical Guidelines: Parenteral Nutrition Ordering, Order Review, Compounding, Labeling, and Dispensing JPEN. 2014; 3(38):334–377.
10. Yalcin S., Gumus M., Oksuzoglu B., Ozdemir F., Evrensel T., Sarioglu A., et al. Nutritional Aspect of Cancer Care in Medical Oncology Patients. *Clinical Therapeutics*. 2019; 41(11):2382-2396.
11. Sioson M., Martindale R., Abayadeera A., Abouchaleh N., Aditianingsih D., Bhurayanontachai R., et al. Nutrition therapy for critically ill patients across the Asia–Pacific and Middle East regions: A consensus statement. *Clinical Nutrition ESPEN*. 2018; 24:156-164.
12. Value of Nutrition Support Therapy: Impact on Clinical and Economic Outcomes in the United States. *Journal of Parenteral and Enteral Nutrition.* 2020; 44(3):395-406.
13. Rollins C., Durfee S.M., Holcombe B.J., et al. Standards of practice for nutrition support pharmacists. *Nutr Clin Pract.* 2008; 23(2):189—94.
14. Holcombe B.J., Thorn D.B., Strausburg K.M., et al. Analysis of the practice of nutrition support pharmacy specialists. *Pharmacotherapy*. 15(6):806—13.
15. Ukleja A., Freeman K.L., Gilbert K., et al. Standards for nutrition support: adult hospitalized patients. *Nutr Clin Pract.* 2010; 25(4):403—14.
16. Pharmaceutical compounding: sterile preparations, the United States pharmacopoeia, 30th rev, and the National Formulary. 25th ed. Rockville MD: *The United States Pharmacopeial Convention*. 2007; 334—51
17. Hilgarth H., Wichmann D., Baehr M., Kluge S. and Langebrake C. Clinical pharmacy services in critical care: results of an observational study comparing ward-based with remote pharmacy services. *Int J Clin Pharm*. 2023; 45(4):847-856. doi:10.1007/s11096-023-01559-z
18. Borthwick M. The role of the pharmacist in the intensive care unit. *J Intensive Care Soc.* 2019; 20(2):161-164. doi:10.1177/1751143718769043
19. Arredondo E., Udeani G., Horseman M., Hintze T.D. and Surani S. Role of Clinical Pharmacists in Intensive Care Units. *Cureus*. 2021; 13(9):e17929. doi:10.7759/cureus.17929
20. Jurado L.V. and Steelman J.D. The role of the pharmacist in the intensive care unit. *Crit Care Nurs Q.* 2013; 36(4):407-414. doi:10.1097/CNQ.0b013e3182a11057
21. Althomali A., Altowairqi A., Alghamdi A., et al. Impact of Clinical Pharmacist Intervention on Clinical Outcomes in the Critical Care Unit, Taif City, Saudi Arabia: A Retrospective Study. *Pharmacy (Basel).* 2022; 10(5):108. doi:10.3390/pharmacy10050108

22. Bosma B.E., van den Bemt P.M.L.A., Melief P.H.G.J., van Bommel J., Tan S.S. and Hunfeld N.G.M. Pharmacist interventions during patient rounds in two intensive care units: Clinical and financial impact. *Neth J Med*. 2018; 76(3):115-124.
23. Fideles G.M., de Alcântara-Neto J.M., Peixoto Júnior A.A., et al. Pharmacist recommendations in an intensive care unit: three-year clinical activities. *Rev Bras Ter Intensiva*. 2015; 27(2):149-154. doi:10.5935/0103-507X.20150026
24. Muñoz-Pichuante D. and Villa-Zapata L. Benefit of Incorporating Clinical Pharmacists in an Adult Intensive Care Unit: A Cost-saving Study. *J Clin Pharm Ther*. 2020; 45(5):1127-1133. doi:10.1111/jcpt.13195
25. Al Dali S., Al-Badriyeh D., Gulied A., et al. Characteristics of the clinical pharmacist interventions at the National Center for Cancer Care and Research Hospital in Qatar. *Journal of Oncology Pharmacy Practice.* 2023; 0(0). doi:10.1177/10781552231187305
26. Alqassieh R., Odeh M., and Jirjees F. Intraoperative Insulin Infusion Regimen versus Insulin Bolus Regimen for Glucose Management during CABG Surgery: A Randomized Clinical Trial. *Jordan Journal of Pharmaceutical Sciences*. 2023; 16(3):487-498. https://doi.org/10.35516/jjps.v16i3.708
27. Daghash R., Al-Saaideh M. A. and Itani R. Evaluation of pharmacotherapy standards during pregnancy among Jordanian pharmacy Colleges graduates. *Jordan Journal of Pharmaceutical Sciences.* 2023; 16(3):607–620. https://doi.org/10.35516/jjps.v16i3.972
28. Jarab A. S., Al-Qerem W., Mukattash T. L., Alqudah S. G., Abu-Zaytoun L., Al-Azayzih A., and Khdour M. Public Perception of Pharmacist's Role during COVID-19 Outbreak in Jordan. *Jordan Journal of Pharmaceutical Sciences*. 2022; 15(3):365–377. https://doi.org/10.35516/jjps.v15i3.410
29. The jamovi project. jamovi. 2022; (Version 2.3) [Computer Software]. Retrieved from https://www.jamovi.org.
30. R Core Team. R: A Language and environment for statistical computing. 2021. (Version 4.1) [Computer software]. Retrieved from https://cran.r-project.org. (R packages retrieved from MRAN snapshot 2022-01-01).
31. Zhou X., Qiu F., Wan D., et al. Nutrition support for critically ill patients in China: role of the pharmacist. *Asia Pac J Clin Nutr*. 2019; 28(2):246-251. doi:10.6133/apjcn.201906_28(2).0006
32. Katoue M.G. and Al-Taweel D. Role of the pharmacist in parenteral nutrition therapy: challenges and opportunities to implement pharmaceutical care in Kuwait. *Pharm Pract (Granada).* 2016; 14(2):680. doi:10.18549/PharmPract.2016.02.680
33. Giancarelli A. and Davanos E. Evaluation of Nutrition Support Pharmacist Interventions. *Journal of Parenteral and Enteral Nutrition*. 2014; 39(4): 476-481.
34. Salman G. and Boullata J.I. The Value of Nutrition Support Pharmacist Interventions. *Crit Care Explor*. 2022; 10(2):e0650. doi:10.1097/CCE.0000000000000650. PMID: 35211684; PMCID: PMC8860336.
35. Gallagher J., Byrne S., Woods N., Lynch D. and McCarthy S. Cost-outcome description of clinical pharmacist interventions in a university teaching hospital. *BMC Health Serv Res*. 2014; 14:177. doi:10.1186/1472-6963-14-177
36. Hammad E.A., Qudah R.A. and Akour A.A. The impact of clinical pharmacists in improving Jordanian patients' health outcomes. *Saudi Med J*. 2017; 38(11):1077-1089. doi: 10.15537/smj.2017.11.21453. PMID: 29114694; PMCID: PMC5767609.
37. Aljbouri T.M., Alkhawaldeh M.S., Abu-Rumman A.E., Hasan T.A., Khattar H.M. and Abu-Oliem A.S. Impact of clinical pharmacist on cost of drug therapy in the ICU. *Saudi Pharm J*. 2013; 21(4):371-374. doi:10.1016/j.jsps.2012.12.004
38. Al-azzam S.I., Shara M., Alzoubi K.H., Almahasneh F.A. and Iflaifel M.H. Implementation of clinical pharmacy services at a university hospital in Jordan. *Int J Pharm Pract*. 2013; 21(5):337-340. doi:10.1111/ijpp.12009
39. Department of Pharmacy, Department of Pharmacy | King Hussein Cancer Foundation and Center. 2024. Available at: https://www.khcc.jo/en/pharmacy.

خدمات صيدلة الدعم الغذائي في مرضى السرطان في وحدات العناية المركزة: تحليل تداخلات الصيادلة السريرين

نادين عبد الهادي[1]*، الاء دبوس[2]، سعد جدوع[2]

[1] كلية الصيدلة، جامعة العقبة للتكنولوجيا، العقبة، الأردن.

[2] قسم الصيدلة، مركز الحسين للسرطان، عمان، الأردن.

ملخص

الأهداف: تهدف هذه الدراسة إلى تحليل تدخلات الصيادلة السريريين في إدارة علاج دعم التغذية في مرضى السرطان المصابين بأمراض خطيرة الذين يتم إدخالهم إلى وحدات العناية المركزة.

الطريقة: تم إجراء تحليل بأثر رجعي ل 9949 تدخلا صيدليا سريريا تم الإبلاغ عنها إلكترونيا للمرضى الذين تم إدخالهم إلى وحدة العناية المركزة من يناير 2020 إلى ديسمبر 2022. تم تضمين وتحليل جميع سجلات المرضى مع تدخلات الصيادلة السريريين المتعلقة بعلاج دعم التغذية في مرضى السرطان في وحدة العناية المركزة.

النتائج: كان متوسط عمر المشاركين في الدراسة 38.1 سنة. وبلغ عدد التدخلات المتعلقة بإدارة العلاج الداعم للتغذية 95 تدخلا. كان متوسط الوقت الذي استغرقه الصيدلي السريري للتدخل 19.2 دقيقة. كانت التغذية الوريدية هي الفئة الأكثر استخداما من علاجات دعم التغذية. كان تقييم وتعديل ومراقبة التغذية الوريدية الكلية هو التدخل الأكثر شيوعا للصيادلة السريريين.

الخلاصة: يلعب الصيادلة السريريون دورا في إدارة العلاج المتخصص لدعم التغذية. كان تدخل الصيادلة السريريين السائد هو تقييم وتعديل ومراقبة التغذية الوريدية الكلية. هناك حاجة إلى مزيد من الدراسات للتحقيق في العوامل التي تؤثر على الوقت اللازم لتوفير تدخلات الصيدلة السريرية عبر مختلف السكان والخدمات الصحية.

الكلمات الدالة: خدمات الصيدلة الإكلينيكية، صيدلية دعم التغذية، السرطان، الرعاية الحرجة.

* ***المؤلف المراسل: نادين عبد الهادي***

nadine_abdelhadi@yahoo.com

تاريخ استلام البحث 2024/04/09 وتاريخ قبوله للنشر 2024/06/14.

A Comprehensive Review on Documentation Practices in the Pharmaceutical Manufacturing Industry

Thanuja. R[1], K. Vinod Kumar[2], Byreddy Divya Sree[2], Karthick. S[2], T. Reshma[1*]

[1]Department Pharmaceutical Quality Assurance, Raghavendra Institute of Pharmaceutical Education and Research, K R Palli Corss, Chiyyedu, Anantapur, Andhra Pradesh, India.
[2]Department Pharmaceutical Analysis, Raghavendra Institute of Pharmaceutical Education and Research, K R Palli Corss, Chiyyedu, Anantapur, Andhra Pradesh, India.

ABSTRACT

This review sheds light on the crucial role of documentation in the pharmaceutical manufacturing industry, a sector where quality control and regulatory compliance are critical. Documentation, from standard operating procedures to validation protocols, underpins quality assurance systems by shaping and improving manufacturing processes. We examine its significance in maintaining product quality, patient safety, and meeting regulatory requirements, particularly in FDA and EMA audits. Additionally, we explore the digital transformation in documentation practices, introducing of electronic batch records, and the associated challenges and opportunities. The paper underscores the necessity for robust, accurate, and timely documentation, emphasizing it as a pledge to product quality, patient safety, and public health.

Keywords: Documentation, Validation Protocols, SOP's, FDA, Pharmaceutical Industry.

1. INTRODUCTION

The pharmaceutical industry is one of the most regulated industries in the world. It has strict regulations and guidelines set by various regulatory agencies such as the FDA, EMA, and WHO, documentation is critical in ensuring that pharmaceutical companies comply with these requirements. The documentation used in the pharmaceutical industry serves several purposes, including supporting research and development, manufacturing, quality control, and distribution of pharmaceutical products. Documentation also plays a crucial role in managing changes in the pharmaceutical industry. Change control documentation includes change requests, change control plans, and change control records, which are used to manage changes to processes, procedures or equipment that may impact the quality or safety of a product. These documents must be maintained and updated regularly to ensure that changes are appropriately managed, and that the product continues to meet regulatory requirements(1).

Validation documentation is also crucial in the pharmaceutical industry. Validation documentation includes validation plans, protocols, and reports, which demonstrate that equipment, processes, and systems used in manufacturing pharmaceutical products consistently produce products that meet established quality standards. The documentation process for validation must be rigorous to ensure that the validation is comprehensive and meets regulatory requirements.

Quality control documentation is also essential in the pharmaceutical industry. These records provide evidence that the product meets the established quality standards. Quality control documentation includes records of all testing and inspections performed on raw materials,

[*]***Corresponding author: T. Reshma***
shaikreshmat614@gmail.com
Received: 11/07/2023 Accepted: 18/07/2024.
DOI: https://doi.org/10.35516/jjps.v17i4.1405

 © 2024 DSR Publishers/The University of Jordan. All Rights Reserved.

intermediates, and finished products.

Regulatory documentation is critical in the pharmaceutical industry. Regulatory documentation includes all records required by regulatory agencies to support product's safety, efficacy, and quality. This includes drug registration dossiers, clinical trial data, and post-marketing surveillance reports. The documentation process for regulatory documents must be thorough and meticulous to ensure that the regulatory requirements are met.

The pharmaceutical industry is one of the most regulated industries in the world, and documentation plays a crucial role in ensuring compliance with various laws, regulations, and guidelines. Documentation in the pharmaceutical industry refers to any written or electronic records created, maintained, and used to support various aspects of pharmaceutical operations, such as research and development, manufacturing, quality control, and distribution. Pharmaceutical industry documentation provides evidence that a product has been developed, manufactured, and distributed according to established quality standards and regulatory requirements. The documentation also helps to ensure that processes are consistent, controlled, and well-documented, reducing the risk of errors, defects, or other quality issues. Effective documentation management is essential in the pharmaceutical industry to ensure that products are safe, effective, and quality. Companies must follow rigorous processes for creating, maintaining, and updating documentation to ensure that it is accurate, complete, and up-to-date. Documentation is also critical for demonstrating compliance with regulatory requirements, such as Good Manufacturing Practices (GMPs) and Good Clinical Practices (GCPs), and for providing evidence to regulatory agencies during product registration and approval processes.

Pharmaceutical industry documentation is essential to pharmaceutical operations, supporting quality, safety, and compliance with regulatory requirements. It plays a critical role in ensuring that pharmaceutical products meet the highest quality and safety standards and that patients receive safe and effective treatments.

Good documentation practice (GDP) is a set of guidelines and standards that govern documentation creation management, and maintenance in various industries. It ensures that documentation is accurate, reliable, and accessible, enabling effective communication and knowledge transfer. Whether it pertains to scientific research, software development, manufacturing processes, or regulatory compliance, adhering to good documentation practices is essential(2).

SOPs are detailed instructions that outline how specific tasks should be performed consistently and standardized. SOPs are critical in ensuring that pharmaceutical companies operate consistently and controlled, helping maintain product quality and safety. The documentation process for SOPs must be rigorous, ensuring that the SOPs are accurate, up-to-date, and followed correctly.

Another essential document in the pharmaceutical industry is batch records. Batch records contain information about manufacturing a particular batch of a drug product. These records include information about raw materials, manufacturing processes, equipment used, and testing results. Batch records are critical in ensuring that the manufacturing process is consistent and that products meet the required specifications(3, 4)

2. TYPES OF DOCUMENTATION

The pharmaceutical industry generates vast documentation to support various aspects of pharmaceutical operations, including research and development, manufacturing, quality control, and distribution. The types of documentation used in the pharmaceutical industry can be broadly categorized as follows(5, 6).

2.1 Standard Operating Procedures (SOPs):

SOPs are detailed written instructions that describing how to consistently and uniformly perform specific tasks.

SOPs are essential in ensuring that pharmaceutical companies operate in a controlled and consistent way, helping to maintain product quality and safety. SOPs cover a wide range of activities, from laboratory testing procedures to manufacturing processes and packaging and labelling instructions. Standard Operating Procedure (SOPs) in the pharmaceutical industry are critical documents that provide detailed instructions for performing specific tasks consistently and uniformly. SOPs are essential in ensuring that pharmaceutical companies operate in a controlled and consistent way, helping to maintain product quality and safety. SOPs are used throughout the pharmaceutical industry for a wide range of activities, including research and development, manufacturing, quality control, and distribution. Some common areas where SOPs are used in the pharmaceutical industry include:

Laboratory Testing Procedures: SOPs define the procedures for testing raw materials, intermediates, and finished products. These procedures ensure that products meet the required quality standards and comply with regulatory requirements.

Manufacturing Processes: SOPs define the processes used to manufacture pharmaceutical products, including equipment, materials, and personnel. These procedures ensure that products are consistently manufactured according to established quality standards.

Packaging and Labelling: SOPs define the procedures used to package and label pharmaceutical products, ensuring that they meet regulatory requirements and are safe and effective for patients.

Cleaning and Maintenance: SOPs define the procedures used to clean and maintain equipment, ensuring that it is in good working order and that products are not contaminated during the manufacturing process. SOPs are typically developed and maintained by subject matter experts in collaboration with quality assurance professionals. The SOP development process typically involves the following steps:

Identify the process or activity that requires an SOP.

Define the scope and purpose of the SOP.

Develop the SOP draft, including detailed instructions and requirements.

Review the SOP draft with subject matter experts and quality assurance professionals.

Revise the SOP as necessary based on feedback and review.

Approve the final SOP and ensure that it is properly communicated and implemented.

Once an SOP is approved, it must be maintained and updated regularly to remain accurate and up-to-date.

This includes reviewing the SOP periodically and updating it as needed to reflect changes in the process, equipment, or regulatory requirements.

The SOPs are essential documents in the pharmaceutical industry, providing detailed instructions for performing specific tasks consistently and uniformly. SOPs help ensure that pharmaceutical products meet established quality standards and comply with regulatory requirements. The SOP development process is thorough and involves subject matter experts and quality assurance professionals to ensure that the SOP is accurate, complete, and effective in supporting the operation(7, 8).

2.1.1 Benefits of SOPs:

Standard Operating Procedures(SOPs)in the pharmaceutical industry are critical documents that provide detailed instructions for how to perform specific tasks consistently and uniformly. SOPs are essential in ensuring that pharmaceutical companies operate in a controlled and consistent way, helping to maintain product quality and safety.

SOPs are used throughout the pharmaceutical industry for a wide range of activities, including research and development, manufacturing, quality control, and distribution. Some common areas where SOPs are used in the pharmaceutical industry include(8, 9)

2.2 Batch Records:

Batch records are documents that provide a detailed

account of the production and testing of a particular batch of a drug product. These records include information about raw materials, manufacturing processes, equipment used, and testing results. Batch records are critical in ensuring that the manufacturing process is consistent and that products meet the required specifications. In the pharmaceutical industry, batch records are essential documents that provide a complete record of the manufacturing process for a specific batch of a pharmaceutical product. Regulatory agencies require batch records to ensure that pharmaceutical products are manufactured according to established procedures and meet the required quality standards. Batch records typically include information on the raw materials used, equipment and processes involved in manufacturing, and the final product specifications. Some of the key information contained in batch records includes(10)

Identification of the Batch: This includes the batch number, product name, and date of manufacture.

Raw Materials: The batch record includes a list of all raw materials used in the manufacturing process, including their specifications, lot numbers, and expiration dates.

Manufacturing Processes: The batch record includes a detailed description of the manufacturing processes used to produce the batch, including any equipment used and the specific procedures followed.

In-Process Controls: The batch record includes information on any in-process controls performed during the manufacturing process to ensure that the product meets the required quality standards.

Finished Product Testing: The batch record includes information on the testing performed on the finished product, including any specifications or standards used for testing and the results of the testing.

Packaging and Labeling: The batch record includes information on the packaging and labeling of the finished product, including any specific requirements or regulations that must be followed.

Quality control personnel typically review and approve batch records before the batch is released for distribution. The batch record serves as a complete history of the manufacturing process for the batch and provides a means of ensuring that the product was manufactured according to established procedures and meets the required quality standards. Batch records must be maintained and retained for a specified period of time, depending on regulatory requirements. The retention period typically ranges from two to five years, depending on the product and the regulatory agency. The batch records are critical documents in the pharmaceutical industry, providing a complete record of the manufacturing process for a specific batch of a pharmaceutical product. Regulatory agencies require batch records to ensure that pharmaceutical products are manufactured according to established procedures and meet the required quality standards. The batch record includes information on the raw materials used, equipment and processes involved in manufacturing, and the final product specifications. Quality control personnel review and approve batch records and must be maintained and retained for a specified period of time, depending on regulatory requirements(11, 12).

2.2.1 Benefits of Batch records:

Batch records play a critical role in ensuring the quality and safety of pharmaceutical products. Here are some of the key benefits of using batch records:

Compliance with Regulations: Batch records are a regulatory requirement for the pharmaceutical industry. Using batch records helps ensure compliance with various regulations and guidelines, including Good Manufacturing Practice (GMP) requirements.

Traceability: Batch records provide a complete history of the manufacturing process for a specific batch of a pharmaceutical product. This allows for traceability of the product, making it possible to identify the source of any quality issues or defects.

Quality Control: Batch records are used to document the quality control measures taken during the

manufacturing process. This ensures that the product meets the required quality standards and is safe for use by patients.

Efficiency: Batch records provide a standardized approach to the manufacturing process, which can improve efficiency and reduce the likelihood of errors or deviations. By using batch records, manufacturers can ensure that tasks are performed consistently and according to established procedures(13).

Continuous Improvement: Batch records can be used as a tool for continuous improvement. By reviewing batch records, manufacturers can identify areas for improvement in the manufacturing process and implement changes to improve efficiency and quality.

Legal Protection: In the event of a product recall or lawsuit, batch records can provide legal protection. They serve as a complete record of the manufacturing process for a specific batch of a pharmaceutical product, giving evidence of compliance with regulations and guidelines.

The batch records are critical documents in the pharmaceutical industry, providing a complete history of the manufacturing process for a specific batch of a pharmaceutical product. Using batch records ensures compliance with regulations, traceability, quality control, efficiency, continuous improvement, and legal protection. The benefits of using batch records include ensuring the quality and safety of pharmaceutical products, improving efficiency, and reducing the likelihood of errors or deviations in the manufacturing process(14).

2.3. Change Control Documentation:

Change control documentation is used to manage changes to processes, procedures, or equipment that may impact the quality or safety of a product. Change control documents include change requests, change control plans, and change control records. These documents must be maintained and updated regularly to ensure that changes are appropriately managed, and that the product meets regulatory requirements. Change control documentation is a critical component of quality management in the pharmaceutical industry. It refers to the process of documenting and managing changes to a product or process. Change control documentation ensure that changes are made in a controlled and systematic manner, with proper consideration of potential risks and impacts(15).

Change control documentation typically includes the following elements:

Change Request: A change request is the initial document that outlines the proposed change. It should include a clear description of the change, the reason for the change, and any potential impacts or risks.

Impact Assessment: An impact assessment is performed to determine the potential effects of the proposed change. This assessment should consider any potential risks to patient safety, product quality, and regulatory compliance.

Risk Assessment: A risk assessment is performed to identify and evaluate any potential risks associated with the proposed change. This assessment should consider the likelihood and severity of potential risks and determine appropriate risk mitigation measures.

Approval Process: The change request, impact assessment, and risk assessment are typically reviewed and approved by a change control board or committee. This approval process ensures that changes are made in a controlled and systematic manner.

Implementation Plan: An implementation plan is developed to outline the steps required to implement the proposed change. This plan should include timelines, responsibilities, and any necessary resources.

Verification and Validation: Verification and validation activities are performed to ensure that the change has been implemented as planned and has no unintended consequences.

Change control documentation is essential for ensuring pharmaceutical product's safety, efficacy, and quality. By documenting and managing changes in a controlled and systematic manner, pharmaceutical companies can

minimize the risks associated with changes and ensure compliance with regulatory requirements. The change control documentation is a critical component of quality management in the pharmaceutical industry. It includes the change request, impact assessment, risk assessment, approval process, implementation plan, and verification and validation. Change control documentation ensures that changes are made in a controlled and systematic manner, with proper consideration of potential risks and impacts. Change control documentation is essential for ensuring the safety, efficacy, and quality of pharmaceutical products and ensuring compliance with regulatory requirements(16).

2.3.1 Benefits of change control:

Change control documentation offers several benefits to the pharmaceutical industry, including:

Ensuring product quality: Change control documentation ensures that changes to the product or process are controlled and systematic, with proper consideration of potential risks and impacts. This helps to minimize the risks associated with changes, ensuring the quality and safety of the final product.

Compliance with regulations: Change control documentation is a regulatory requirement for the pharmaceutical industry. By documenting and managing changes in a controlled manner, pharmaceutical companies can ensure compliance with various regulations and guidelines, including Good Manufacturing Practice (GMP) requirements.

Enhancing efficiency: Change control documentation can also help enhance efficiency in pharmaceutical manufacturing. By documenting changes and their impacts, manufacturers can identify and implement improvements that increase efficiency and reduce costs.

Facilitating communication: Change control documentation provides a clear and concise record of changes, which can facilitate communication between different departments within a pharmaceutical company. This ensures that everyone is aware of changes and can take the necessary actions to implement them.

Minimizing risks: Change control documentation helps to minimize the risks associated with changes, ensuring that changes are properly assessed, planned, and implemented. This reduces the likelihood of errors, deviations, and product recalls, minimizing risks to patient safety and reducing financial and reputational risks for the pharmaceutical company.

The change control documentation is a critical tool for ensuring the pharmaceutical product's quality, safety, a compliance. It offers several benefits, including ensuring product quality, compliance with regulations, enhancing efficiency, facilitating communication, and minimizing risks. By documenting and managing changes controlled a systematically, pharmaceutical companies can improve their operations, protect patient safety, and maintain regulatory compliance(17, 18).

2.4. Validation Documentation:

Validation documentation is used to demonstrate that equipment, processes, and systems used in the manufacture of pharmaceutical products are consistently producing products that meet established quality standards. Validation documentation includes validation plans, protocols, and reports.

Validation documentation is a crucial component of quality management in the pharmaceutical industry. It refers to the process of documenting and verifying that a pharmaceutical product or process meets its intended use and regulatory requirements. Validation ensures that a product or process is safe, effective, and consistent in its performance(19).

Validation documentation typically includes the following elements:

Validation Plan: A validation plan outlines the approach and methodology for validating a product or process. This plan should include the scope of validation, validation activities to be performed, acceptance criteria, and any necessary resources.

Risk Assessment: A risk assessment identifies a

evaluates potential risks associated with the product or process. This assessment should consider the likelihood and severity of potential risks and determine appropriate risk mitigation measures.

Installation Qualification (IQ): IQ involves documenting and verifying that all equipment, software, and utilities used in the product or process are installed correctly and meet the intended specifications.

Operational Qualification (OQ): OQ involves documenting and verifying that the product or process performs as intended under normal operating conditions. This includes testing and verifying that the equipment and processes are consistent with their intended use.

Performance Qualification (PQ): PQ involves documenting and verifying that the product or process consistently meets its acceptance criteria and performs as intended under a range of operating conditions.

Design Qualification (DQ): Design qualification (DQ) is a process used to ensure that a design meets the required standards and specifications for a particular product or system. It is a documented process that establishes the design inputs, design outputs, and the verification and validation activities necessary to ensure that the design meets the intended use and user needs.

Validation Report: A validation report summarizes the results of the validation activities and documents any deviations or corrective actions taken.

Validation documentation is essential for ensuring pharmaceutical product's quality, safety, and efficacy. By documenting and verifying that a product or process meets its intended use and regulatory requirements, pharmaceutical companies can minimize the risks associated with product failures, protect patient safety, and ensure compliance with regulatory requirements. The validation documentation is a critical component of quality management in the pharmaceutical industry. It includes a validation plan, risk assessment, installation qualification, operational qualification, performance qualification, and validation report. Validation documentation ensures that a product or process meets its intended use and regulatory requirements, minimizing the risks associated with product failures and ensuring the safety, efficacy, and consistency of pharmaceutical products. Validation documentation is essential for maintaining regulatory compliance and protecting patient safety(20).

2.4.1 Benefits of validation documentation:

Validation documentation offers several benefits to the pharmaceutical industry, including:

Ensuring product quality and safety: Validation documentation ensures that pharmaceutical products are manufactured and tested according to the intended specifications and that they meet the required quality and safety standards. This helps to minimize the risks associated with product failures, protecting patient safety and health.

Compliance with regulatory requirements: Validation documentation is a regulatory requirement for the pharmaceutical industry. By documenting and validating manufacturing processes, pharmaceutical companies can ensure compliance with various regulations and guidelines, including Good Manufacturing Practice (GMP) requirements.

Improving efficiency: Validation documentation can also help to improve pharmaceutical manufacturing processes' efficiency. By identifying and addressing potential issues or deviations during the validation process, companies can improve their processes, reduce the risk of errors, and increase productivity.

Facilitating communication: Validation documentation provides a clear and concise record of the manufacturing processes and the testing conducted, which can facilitate communication between different departments within a pharmaceutical company. This ensures that everyone is aware of the validation status and can take the necessary actions to implement improvements or address any issues.

Reducing costs: Validation documentation can help to reduce costs associated with manufacturing, testing, and

product failures. By identifying and addressing potential issues or deviations during the validation process, companies can reduce the likelihood of product failures, minimize waste, and improve the overall efficiency of the manufacturing process.

The validation documentation is essential for ensuring pharmaceutical product's quality, safety and efficacy. It offers several benefits, including ensuring product quality and safety, compliance with regulatory requirements, improving efficiency, facilitating communication, and reducing costs. By documenting and validating manufacturing processes, pharmaceutical companies can ensure the safety and efficacy of their products, protect patient health, and maintain regulatory compliance(20-22)

2.5 Quality Control Documentation:

Quality control documentation provides evidence that the product meets established quality standards. Quality control documentation includes records of all testing and inspections performed on raw materials, intermediates, and finished products.

Quality control documentation is essential to the pharmaceutical industry's quality management system. It refers to the process of documenting and verifying that pharmaceutical products and processes meet the required quality standards. Quality control documentation typically includes the following elements:

Standard Operating Procedures (SOPs): SOPs are written instructions that outline the steps required to perform a specific task or operation. SOPs are used to ensure that processes are performed consistently and according to the intended specifications.

Batch Records: Batch records document the manufacturing and testing activities performed for a specific batch of a pharmaceutical product. Batch records include information on raw materials, manufacturing processes, testing results, and any deviations or corrective actions.

Stability Data: Stability data documents the stability and shelf-life of a pharmaceutical product. Stability data is used to establish expiry dates for the product and to ensure that the product maintains its intended quality over time.

Analytical Methods and Results: Analytical methods and results document the testing methods and results used to verify the quality of a pharmaceutical product. This includes testing for purity, potency, and quality attributes.

Change Control Records: Change control records document any changes made to the product or process and the corresponding impact on product quality. Change control records ensure that changes are made in a controlled and documented manner, minimizing the risks associated with product failures.

Deviation and Investigation Reports: Deviation and investigation reports document any deviations from established procedures or specifications and the corresponding investigation and corrective action taken.

Quality control documentation is critical for ensuring that pharmaceutical products meet the required quality standards and comply with regulatory requirements. By documenting and verifying manufacturing processes, testing results, and any deviations or corrective actions taken, pharmaceutical companies can ensure that their products are safe, effective, and of high quality. The quality control documentation is a crucial component of the pharmaceutical industry's quality management system. It includes standard operating procedures, batch records, stability data, analytical methods and results, change control records, and deviation and investigation reports. Quality control documentation ensures that pharmaceutical products meet the required quality standards, comply with regulatory requirements, and are safe, effective, and of high quality. By documenting and verifying manufacturing processes, testing results, and any deviations or corrective actions taken, pharmaceutical companies can maintain regulatory compliance, protect patient health, and maintain their reputation for producing high-quality pharmaceutical products (2).

2.5.1 Benefits of quality control documentation:

Quality control documentation provides several benefits to the pharmaceutical industry, including:

Ensuring product quality and safety: Quality control documentation ensures that pharmaceutical products meet the required quality standards and are safe and effective for use. This helps to protect patient health and minimize the risks associated with product failures.

Compliance with regulatory requirements: Quality control documentation is a regulatory requirement for the pharmaceutical industry. Pharmaceutical companies can ensure compliance with various regulations and guidelines, including Good Manufacturing Practice (GMP) requirements by documenting and verifying manufacturing processes, testing results, and any deviations or corrective actions taken.

Identifying and addressing issues: Quality control documentation helps to identify any issues or deviations from established procedures or specifications. By documenting and investigating these issues, companies can take corrective action to address the root cause of the problem and prevent it from happening again in the future.

Improving efficiency: Quality control documentation can also help to improve pharmaceutical manufacturing processes' efficiency. By identifying and addressing potential issues or deviations during the quality control process, companies can improve their processes, reduce the risk of errors, and increase productivity.

Facilitating communication: Quality control documentation provides a clear and concise record of the manufacturing processes and the testing conducted, which can facilitate communication between different departments within a pharmaceutical company. This ensures that everyone is aware of the quality control status and can take the necessary actions to implement improvements or address any issues.

Reducing costs: Quality control documentation can help to reduce costs associated with manufacturing, testing, and product failures. By identifying and addressing potential issues or deviations during the quality control process, companies can reduce the likelihood of product failures, minimize waste, and improve the overall efficiency of the manufacturing process.

The quality control documentation is critical for ensuring the quality, safety, and efficacy of pharmaceutical products. It offers several benefits, including ensuring product quality and safety, compliance with regulatory requirements, identifying and addressing issues, improving efficiency, facilitating communication, and reducing costs. By documenting and verifying manufacturing processes, testing results, and any deviations or corrective actions taken, pharmaceutical companies can ensure the safety and efficacy of their products, protect patient health, and maintain regulatory compliance (3)(22).

2.6. Regulatory Documentation:

Regulatory documentation includes all records required by regulatory agencies to support product's safety, efficacy, and quality. This includes drug registration dossiers, clinical trial data, and post-marketing surveillance reports. The documentation process for regulatory documents must be thorough and meticulous to ensure that the regulatory requirements are met.

Regulatory documentation essential to the pharmaceutical industry's quality management system. It refers to the documentation required to ensure compliance with regulatory requirements, including those set by government agencies such as the US Food and Drug Administration (FDA) or the European Medicines Agency (EMA). Regulatory documentation includes the following types of documents:

Investigational New Drug (IND) Application: An IND application is submitted to the FDA to seek approval to conduct clinical trials on a new drug or biological product. It includes information on the drug's chemistry, manufacturing, and controls (CMC), and preclinical and clinical data.

New Drug Application (NDA) or Biologics License Application (BLA): An NDA or BLA is submitted to the FDA to seek approval to market a new drug or biological product. It includes detailed information on the drug's

safety, efficacy, and quality, as well as data from clinical trials and CMC information.

Marketing Authorization Application (MAA): An MAA is submitted to the EMA to seek approval to market a new drug or biological product in the European Union. It includes data on the drug's quality, safety, and efficacy, as well as clinical trial data and CMC information.

Drug Master File (DMF): A DMF is a confidential document submitted to regulatory agencies that contains detailed information on the manufacturing, processing, packaging, and testing of a drug substance or product. It is used to support the regulatory approval of a drug product or to provide information to regulatory agencies about the quality of the drug substance.

Annual Reports: Annual reports provide updates on the safety and efficacy of a drug product or biological product, as well as any changes to the manufacturing process or labeling.

Adverse Event Reports: Adverse event reports document any adverse events or side effects associated with the use of a drug product. These reports are used to evaluate the safety of the product and to identify any potential safety concerns.

Regulatory documentation is critical for ensuring compliance with regulatory requirements and obtaining approval to market pharmaceutical products. By providing detailed information on the drug's safety, efficacy, and quality, as well as data from clinical trials and CMC information, regulatory documentation helps to ensure that pharmaceutical products are safe and effective for use. It also helps to protect patient health and minimize the risks associated with product failures. The regulatory documentation is a crucial component of the pharmaceutical industry's quality management system. It includes documents such as IND applications, NDAs/BLAs, MAAs, DMFs, annual reports, and adverse event reports. Regulatory documentation is essential for obtaining approval to market pharmaceutical products and ensuring compliance with regulatory requirements. By providing detailed information on the safety, efficacy, and quality of pharmaceutical products, regulatory documentation helps to protect patient health, minimize the risks associated with product failures, and maintain the reputation of the pharmaceutical industry.8bt,8y(23).

2.6.1 Benefits of regulatory documentation:

Regulatory documentation provides several benefits to the pharmaceutical industry, including:

Compliance with regulatory requirements: Regulatory documentation is necessary for obtaining regulatory approval to market pharmaceutical products. By providing detailed information on the product's safety, efficacy, and quality, regulatory documentation helps to ensure that the product meets the regulatory requirements set by government agencies such as the FDA and EMA.

Improved product quality: Regulatory documentation provides detailed information on the manufacturing, processing, packaging, and testing of a drug product. This information helps to ensure that the product is of high quality, and that it is safe and effective for use.

Increased patient safety: Regulatory documentation includes adverse event reports, which document any adverse events or side effects associated with the use of a drug product. This information helps to evaluate the safety of the product and identify any potential safety concerns. By addressing safety concerns promptly, the industry can improve patient safety.

Protection of public health: Regulatory documentation helps to protect public health by ensuring that pharmaceutical products are safe and effective for use. By providing detailed information on the product's safety, efficacy, and quality, regulatory documentation helps minimize the risks associated with product failures.

Improved industry reputation: Regulatory documentation helps maintain the pharmaceutical industry's reputation by ensuring that products are of high quality, safe, and effective for use. By complying with regulatory requirements and providing detailed

information on the safety, efficacy, and quality of products, the industry can build and maintain trust with patients and healthcare providers.

The regulatory documentation is essential for the pharmaceutical industry. It provides several benefits, including compliance with regulatory requirements, improved product quality, increased patient safety, protection of public health, and improved industry reputation. By investing in regulatory documentation, the industry can ensure that it meets the highest standards of quality and safety, and continue to provide patients with safe and effective products(9, 24)

2.7. Test Methods and Specifications:

These documents specify the tests that need to be conducted to ensure that the drug product meets the required quality and safety standards. Test methods and specifications are essential components of the software development process. They are used to ensure that software products meet certain quality standards, are free of defects, and perform as expected. Test methods are techniques that are used to verify that a software product meets its intended functionality and performance requirements.

These methods can include automated and manual tests, performance tests, regression tests, and usability tests, among others. The choice of test methods will depend on the nature of the software product and the specific requirements it needs to meet.

Test specifications are documents that define the requirements and procedures for testing a software product. They provide a detailed description of the expected behavior of the software product and the criteria that will be used to determine whether it meets those expectations. Test specifications can include information such as test cases, test scripts, test data, and acceptance criteria.

Developing test methods and specifications is an iterative process that involves collaboration between software developers, testers, and other stakeholders. The process begins with the identification of the software requirements, which are then used to define the test specifications. Test methods are then developed based on the specifications, and the software product is tested using those methods. Any defects or issues that are found during testing are then addressed, and the testing process is repeated until the software product meets the required quality standards. The test methods and specifications are critical to the success of any software development project. They help to ensure that software products are of high quality, meet customer expectations, and are free of defects. Using automated and manual tests, performance tests, regression tests, and usability tests, software developers can ensure that their products are reliable, secure, and perform as expected(25).

2.7.1 Benefits of test method specifications:

Test methods and specifications offer several benefits in various fields and industries. Here are some key advantages:

Standardization: Test methods and specifications provide standardized procedures and criteria for evaluating and measuring various parameters. They establish a common framework that ensures consistency and comparability of results across different organizations, laboratories, or testing facilities. Standardization facilitates effective communication, collaboration, and quality control.

Quality Control and Assurance: Test methods and specifications enable organizations to maintain consistent quality levels for their products or processes. By defining specific requirements and performance criteria, these methods help identify deviations or deficiencies, allowing for timely corrective actions. They play a vital role in quality control, ensuring that products meet desired standards and customer expectations.

Product Development and Design: Specifications provide clear guidelines and requirements during the product development or design phase. They help establish the necessary characteristics, features, and performance

levels a product should meet. Test methods complement this by providing techniques and procedures to verify and validate whether the product satisfies the specified requirements.

Compliance and Regulation: Test methods and specifications assist organizations in complying with industry regulations, safety standards, and legal requirements. They ensure that products meet the necessary safety, performance, and quality benchmarks. Compliance with these standards helps organizations avoid penalties, litigation, and reputational damage, while ensuring end-users safety and satisfaction.

Cost Reduction and Efficiency: Test methods and specifications aid in identifying and eliminating inefficiencies, redundancies, or unnecessary features in products or processes. By specifying only essential requirements, organizations can optimize their resources, reduce manufacturing costs, and enhance overall operational efficiency. Additionally, standardized test methods enable faster and more efficient testing processes, saving time and resources.

Risk Mitigation: Test methods and specifications help in identifying and mitigating risks associated with products or processes. By defining safety and performance criteria, organizations can assess and manage potential risks more effectively. Robust testing procedures identify and address potential hazards, weaknesses, or failure points, reducing the likelihood of accidents, malfunctions, or safety incidents.

Customer Satisfaction and Market Competitiveness: Test methods and specifications contribute to customer satisfaction by ensuring that products meet or exceed their expectations. Organizations can deliver consistent quality, reliability, and performance by adhering to defined specifications, enhancing customer trust and loyalty. Meeting or surpassing industry standards and specifications also helps organizations stay competitive in the market.

Research and Development: Test methods and specifications support research and development activities by providing a framework for evaluating new ideas, technologies, or prototypes. They enable researchers to establish benchmarks, measure performance, and compare results against predefined criteria. This fosters innovation, accelerates progress, and promotes advancements in various fields.

Test methods and specifications bring structure, consistency, and reliability to the evaluation, development, and manufacturing processes. They ensure quality, compliance, and customer satisfaction while supporting innovation and risk mitigation(26, 27).

2.8. Protocols and Reports:

These documents outline the procedures and results of tests and experiments conducted during the drug development process.

Stability Studies: These documents describe the stability testing performed on the drug product to ensure that it remains safe and effective throughout its shelf-life.

Protocols and reports are important documents that are used in a variety of fields, including medicine, science, engineering, and business. They provide detailed information about procedures, experiments, and projects, and are essential for ensuring accuracy and reproducibility. A protocol is a document that describes the procedures and methods that will be used in an experiment or project. It that will be used, the measurements that will be taken, and the analysis that will be performed. Protocols are essential for ensuring that experiments and projects are carried out in a consistent and reproducible manner, and for minimizing the risk of errors and inaccuracies. In science and medicine, protocols are used to ensure that experiments and clinical trials are conducted in a standardized manner, so that results can be compared and replicated. In engineering and business, protocols are used to ensure that projects are carried out efficiently and effectively, and that they meet the required quality standards. A report is a document that provides a detailed analysis of the results of an experiment or project. It

typically includes an introduction that provides background information, a methodology section that describes the procedures used, a results section that presents the data and findings, and a discussion section that interprets the results and draws conclusions. Reports are essential for communicating the results of experiments and projects to stakeholders, including funding agencies, regulatory bodies, and other researchers.

In science and medicine, reports are used to communicate the results of research studies and clinical trials to other researchers, healthcare professionals, and the public. In engineering and business, reports are used to communicate the results of projects to clients, stakeholders, and other members of the project team. The protocols and reports are essential documents in a variety of fields, including medicine, science, engineering, and business. Protocols provide a standardized method for carrying out experiments and projects, while reports communicate the results of those experiments and projects to stakeholders. Together, they help to ensure accuracy, reproducibility, and accountability in research, development, and project management(28).

2.8.1 Benefits of Protocols:

Clear Experimental Design: Protocols outline the step-by-step procedures and methodologies to be followed in an experiment or study. They provide a clear structure and guidance, ensuring that experiments are conducted consistently and accurately. Well-defined protocols help eliminate ambiguity and increase the reliability of research outcomes.

Reproducibility: Protocols facilitate reproducibility by providing detailed instructions that allow other researchers or teams to replicate the study. Reproducibility is critical for validating research findings, building upon previous work, and advancing scientific knowledge.

Standardization: Protocols contribute to standardization within research or operational workflows. They establish uniform approaches and techniques, ensuring consistency across different experiments or projects. Standardization improves collaboration, facilitates data sharing, and promotes comparability of results.

Quality Control: Protocols serve as quality control tools by defining the necessary checks, controls, and measurements at each stage of an experiment or process. They help identify potential sources of error, deviations, or biases, ensuring that data and results are reliable and accurate.

Regulatory Compliance: In regulated industries, protocols ensure compliance with legal, ethical, and safety requirements. They provide guidelines on handling sensitive data, maintaining privacy, adhering to ethical principles, and ensuring participant or subject safety. compliance with protocols helps organizations meet regulatory standards and avoid legal or ethical issues(29).

2.8.2 Benefits of Reports:

Documentation: Reports serve as comprehensive records of research findings, project progress, or operational activities. They document methodologies, results, and conclusions, providing a reference for future analysis, decision-making, or audits. Reports ensure that information is preserved and accessible over time.

Communication and Knowledge Sharing: Reports enable effective communication of research findings, project outcomes, or operational insights to stakeholders. They provide a concise summary of the work done, allowing others to understand and build upon the results. Reports foster knowledge- sharing, collaboration, and informed decision-making.

Evaluation and Analysis: Reports provide a framework for analyzing and evaluating data, results, or performance. They allow for critical examination, interpretation, and comparison of findings against predefined objectives or benchmarks. Reports facilitate data-driven decision-making and contribute to continuous improvement.

Accountability and Transparency: Reports promote accountability by documenting and disclosing the details

of research or operational activities. They provide transparency, allowing others to scrutinize the processes, results, and conclusions. Transparent reporting enhances trust, credibility, and integrity in scientific research, business operations, or project management.

Archiving and References: Reports serve as valuable references for future research, replication, or reference purposes. They contribute to collective knowledge and serve as historical records of scientific discoveries, project outcomes, or business performance. Archiving reports ensures that information is preserved and available for future use.

Lessons Learned and Improvement: Reports facilitate the identification of lessons learned from research, projects, or operations. By analyzing successes, challenges, and areas for improvement, organizations can enhance their processes, strategies, and decision-making. Reports provide a basis for continuous learning and improvement.

The protocols and reports provide structure, clarity, and documentation in various domains. They ensure experimental rigor, facilitate reproducibility, support compliance, enable effective communication, and contribute to knowledge sharing and improvement(30).

2.9. Quality Agreements:

These documents outline the responsibilities of all parties involved in the manufacturing, testing, and distribution of the drug product, including the quality standards that need to be met. Quality agreements are a critical component of any successful business relationship involving the manufacturing and supply of pharmaceutical products. These agreements define the roles and responsibilities of the parties involved in the manufacturing process and provide a framework for ensuring product quality, safety, and efficacy. Quality agreements are typically developed between a pharmaceutical company and its contract manufacturing organization (CMO), but they can also be used between a pharmaceutical company and its suppliers, distributors, or even regulatory agencies. They serve as a legally binding document that outlines the quality expectations, processes, and controls required for the production of pharmaceutical products. The purpose of a quality agreement is to ensure that all parties involved in the manufacturing process are aligned in terms of quality expectations and processes. This agreement typically includes sections that outline the responsibilities of each party, the quality control measures that will be implemented, and the processes for handling deviations or quality issues.

Some key elements of a quality agreement include

Scope: The agreement should clearly define the scope of the manufacturing activities and the products that will be produced.

Roles and Responsibilities: The agreement should clearly define the roles and responsibilities of each party involved in the manufacturing process, including the quality control responsibilities of each party.

Quality Standards: The agreement should specify the quality standards that will be followed, including specifications for raw materials, intermediate products, and finished products.

Change Control: The agreement should outline the process for making changes to the manufacturing process, including how changes will be communicated and how they will be evaluated for impact on product quality.

Quality Control Measures: The agreement should outline the quality control measures that will be implemented, including testing requirements, sampling plans, and acceptance criteria.

Deviations and Investigations: The agreement should specify the process for handling deviations or quality issues, including how they will be investigated, documented, and resolved.

Documentation and Records: The agreement should specify the documentation and record-keeping requirements, including the format and retention period for records.

In conclusion, quality agreements are essential for

ensuring that pharmaceutical products are manufactured and supplied in accordance with the required quality standards. By clearly defining the roles and responsibilities of all parties involved in the manufacturing process, quality agreements provide a framework for ensuring product quality, safety, and efficacy, which is crucial for protecting patient health and safety.

Stability studies are an essential part of the pharmaceutical development process, designed to determine a drug products shelf life and storage conditions of a drug product. These studies provide critical information for regulatory submissions and help ensure the drug product's safety, efficacy, and quality throughout its intended shelf life. Stability studies involve monitoring the physical, chemical, and microbiological properties of a drug product over time, under various storage conditions. These conditions can include temperature, humidity, light exposure, and packaging configurations. The studies are typically conducted in accordance with guidelines set by regulatory agencies such as the International Council for Harmonisation (ICH) or the United States Pharmacopeia (USP).

The primary objectives of stability studies are to:

Establish the shelf life and storage conditions of the drug product: Stability studies help to determine the length of time a drug product remains safe and effective under various storage conditions.

Evaluate the effects of environmental factors on the drug product: Stability studies help to identify the potential impact of environmental factors such as temperature, humidity, and light exposure on the drug product.

Verify the quality and consistency of the drug product: Stability studies help ensure that the product remains consistent in terms of quality, potency, and purity over time.

Support regulatory submissions: Stability studies are required for regulatory submissions and are used to demonstrate the safety, efficacy, and quality of the drug product.

Stability studies typically involve testing at various time points, ranging from months to years, depending on the intended shelf life of the drug product. Testing can include physical and chemical tests such as appearance, pH, and dissolution, as well as microbiological tests for sterility or endotoxin levels (9)

The results of stability studies are used to establish the expiration date and storage conditions of the drug product. If stability data indicates that the drug product may be unstable under certain storage conditions, additional packaging or formulation changes may be required to ensure stability.

The stability studies are critical for the development and commercialization of pharmaceutical products. They provide essential information on the shelf life and storage conditions of a drug product, helping to ensure its safety, efficacy, and quality over time. Stability data is also a key component of regulatory submissions and is used to support marketing approvals for new drug products(31).

2.9.1 Benefits of quality agreements:

Quality agreements play a crucial role in establishing clear expectations, responsibilities, and requirements between parties involved in the manufacturing, distribution, or supply of products or services. Here are some benefits of quality agreements:

Clear Communication: Quality agreements provide a platform for clear and effective communication between different entities involved in a business relationship. They outline expectations, specifications, and quality requirements, ensuring that all parties understand their roles, responsibilities, and obligations. Clear communication helps avoid misunderstandings, conflicts, and potential quality issues.

Quality Control and Assurance: Quality agreements establish a framework for quality control and assurance throughout the supply chain. They define quality standards, testing methodologies, and specifications for raw materials, intermediate products, and finished goods. By outlining quality control procedures, sampling plans,

and acceptance criteria, quality agreements help ensure consistent product quality and compliance with regulatory requirements.

Risk Mitigation: Quality agreements help mitigate risks associated with product quality, safety, or regulatory compliance. They identify potential risks and establish mechanisms for their management and mitigation. By outlining processes for change control, deviation management, and non-conformance resolution, quality agreements enable timely and effective risk mitigation measures.

Regulatory Compliance: Quality agreements support regulatory compliance by outlining the responsibilities of each party regarding adherence to applicable regulations and standards. They help ensure that products or services meet regulatory requirements, labeling standards, and documentation obligations. Compliance with quality agreements can streamline regulatory audits and inspections, reducing the risk of non-compliance penalties or delays.

Supplier Relationship Management: Quality agreements are especially beneficial in supplier-customer relationships. They foster a collaborative and transparent relationship between suppliers and customers by clearly defining expectations, quality requirements, and performance metrics. Quality agreements facilitate effective supplier selection, performance evaluation, and ongoing supplier relationship management.

Continuous Improvement: Quality agreements provide a platform for continuous improvement initiatives. They establish mechanisms for feedback, performance monitoring, and periodic review of quality-related activities. Through regular review and evaluation, quality agreements enable identification of improvement opportunities, process optimization, and enhanced product quality.

Contractual Support: Quality agreements provide a contractual basis for quality-related terms and conditions. They define specific quality requirements, acceptance criteria, and dispute resolution mechanisms. Quality agreements support legal recourse in case of quality-related issues, ensuring that the parties involved have a mutually agreed-upon framework for dispute resolution.

Customer Satisfaction and Brand Protection: By ensuring consistent product quality, safety, and compliance, quality agreements contribute to customer satisfaction. Consistently meeting or exceeding customer expectations builds trust and loyalty. Quality agreements help protect Organizations' reputation and brand image by minimizing the risk of quality issues, product recalls, or negative customer experiences.

The quality agreements provide a structured and collaborative approach to ensure consistent product quality, regulatory compliance, and effective supplier relationship management. They facilitate clear communication, risk mitigation, and continuous improvement, ultimately enhancing customer satisfaction and protecting brand reputation(32).

CONCLUSION

In conclusion, the review has elucidated documentation's central role in the pharmaceutical manufacturing industry. It has underscored that documentation is not merely a regulatory obligation but a cornerstone of quality control, patient safety, and public health. Our exploration of different types of documentation, from standard operating procedures to validation protocols, has demonstrated their integral role in creating, controlling, and improving manufacturing processes. The importance of documentation in meeting the stringent regulatory demands of bodies like the FDA and EMA has also been highlighted, emphasizing its role in audit preparedness and regulatory compliance.

Notably, the digital transformation in documentation practices has opened new avenues for efficiency and accuracy. However, it also presents challenges in terms of data security and system validation that must be diligently addressed. The pharmaceutical industry must continue to

prioritize rigorous documentation practices. With the advent of digital tools, this area has the potential for significant improvements. However, these must be balanced with the need for thoroughness, accuracy, and compliance to maintain the highest standards of product quality and patient safety. As this review has shown, robust documentation is not just a functional necessity - it is a commitment to excellence in pharmaceutical manufacturing.

Abbreviations

FDA : Food and drug administration
EMA : European Medicines Agency
WHO : World health organization
GMPs : Good Manufacturing Practices
GCPs : Good Clinical Practices
GDP : Good documentation practice
SOPs : Standard Operating Procedures
ICH : International Council for Harmonisation
USP : United States of Pharmacopeia

Acknowledgement

Authors are extremely thankful to Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh-India.

Conflict of Interest

The authors do not have any conflict of interest.

REFERENCES

1. Sreelekha S., Kumar K.V., Mahammed N., Reshma T., Sree G.U., Basha S.S. and Bhuvaneswari M. Quality by Design Approaches in Pharmaceutical Development and Solid Dosage Forms Production: A Narrative Overview. *Jordan Journal of Pharmaceutical Sciences*. 2023; 16(4): 770-84.
2. Kumar K. Good Documentation Practices (GDPs) in Pharmaceutical Industry. *Journal of Analytical & Pharmaceutical Research*. 2017; 4(2):00100.
3. Simonovski N. and Gjorgjeska B. Documentation as an integral part of quality assurance in the pharmaceutical industry. *Knowledge–International Journal.* 2022; 54(4): 807-11.
4. Ventegodt S. M. and Merrick J. M. The pharmaceutical industry and documentation. *Journal of Alternative Medicine Research.* 2011; 3(4):453.
5. Aghajani E., Nagy C., Vega-Márquez O.L., Linares-Vásquez M., Moreno L., Bavota G., Lanza M. and editors. Software documentation issues unveiled. 2019 IEEE/ACM 41st International Conference on Software Engineering (ICSE). 2019; IEEE.
6. Bloswick A. and Skowron H.A. Informed consent documentation in pharmaceutical industry-sponsored clinical trials. Ethics & Medicine: *An International Journal of Bioethics*. 2015; 31(1):51-9.
7. Jiang X., Zhou Y., Ming W., Yang P. and Wu J. An overview of soft open points in electricity distribution networks. *IEEE Transactions on Smart Grid*. 2022.
8. Figer B.H., Sapra K.P., Gogtay N.J. and Thatte U.M. A comparative study to evaluate quality of data documentation between investigator-initiated and pharmaceutical industry-sponsored studies. *Perspectives in clinical research*. 2020; 11(1):13.
9. Kolekar P. and Bhagwat A. Good documentation practices: a need of pharmaceutical industry. *Asian Journal of Research in Chemistry*. 2021; 368-374. https: //doi.org/10.52711/0974-4150.2021.00063.
10. Serge S. and Serracino-Inglott A. *Standard operating procedures in pharmaceutical quality systems*. 2012.
11. Salvarina I., Gravier D. and Rothhaupt K.O. Seasonal bat activity related to insect emergence at three temperate lakes. *Ecology and Evolution*. 2018; 8(7):3738-50.
12. Orwa J., Keter L., Ouko S., and Kibwage I. Influence of manufacturing practices on quality of pharmaceutical products manufactured in kenya. *East African Medical Journal*. 2004; 81(6). https: //doi.org/10.4314/eamj.v81i6.9177.
13. Mousa M., Hilal Y. and Swed A. Determination of iron in liposomal dosage forms by flame atomic absorption spectrometry after an acidic digestion. *Jordan Journal of Pharmaceutical Sciences.* 2020; 13(4).

14. Huang J., Serra T., Garcia P. and Irwin S.H. To batch or not to batch? The release of USDA crop reports. *Agricultural Economics*. 2022; 53(1):143-54.
15. Alkhatib H., Barqawi A. and Alhawmdeh E. Effect of casein incorporation on the release of diltiazem HCl from hypromellose-based matrix tablets. *Jordan Journal of Pharmaceutical Sciences*. 2018; 11(2).
16. Stasis A., Whyte J. and Dentten R. A critical examination of change control processes. *Procedia CIRP*. 2013; 11:177-82.
17. Bradley G. Benefit Realisation Management: A practical guide to achieving benefits through change: *CRC Press*. 2016.
18. Masli A., Peters G.F., Richardson V.J. and Sanchez J.M. Examining the potential benefits of internal control monitoring technology. *The Accounting Review*. 2010; 85(3):1001-34.
19. Torkzadeh G. The Quality of User Documentation an Instrument Validation. *Journal of management information systems*. 1988; 5(2):99-108.
20. Allenet B., Bedouch P., Rose F-X., Escofier L., Roubille R., Charpiat B., et al. Validation of an instrument for the documentation of clinical pharmacists' interventions. *Pharmacy World and Science*. 2006; 28:181-8.
21. Agalloco J.. Why Validation? Handbook of Validation in Pharmaceutical Processes: *CRC Press*. 2021; 1-4.
22. Bower J., McClung J., Watson C., Osumi T., and Pastre K. Recommendations and best practices for reference standards and reagents used in bioanalytical method validation. *the Aaps Journal*. 2014; 16(2):352-356. https: //doi.org/10.1208/s12248-014-9566-y
23. Carlucci S., Bai L., de Dear R. and Yang L. Review of adaptive thermal comfort models in built environmental regulatory documents. *Building and Environment*. 2018; 137:73-89.
24. Glick S., Guggemos A., editors. IPD and BIM: benefits and opportunities for regulatory agencies. Proc, *45th Associated Schools of Construction National Conference*. 2009.
25. Harrigan E., Leahy R. and Youtcheff J. Superpave manual of specifications, test methods and practices. SHRP-A-379. *Strategic Highway Research Program*. 1994.
26. Hall P., editor Towards testing with respect to formal specification. Second IEE/BCS Conference: Software Engineering, 1988 Software Engineering 88. *IET*. 1988.
27. Narayana S., Pati R., and Vrat P. Research on management issues in the pharmaceutical industry: a literature review. *International Jogdpurnal of pharmaceutical and Healthcare Marketing*. 2012; 6(4):351-375. https: //doi.org/10.1108/17506121211283235.
28. Butcher N.J., Mew E.J., Monsour A., Chan A-W., Moher D. and Offringa M. Outcome reporting recommendations for clinical trial protocols and reports: a scoping review. *Trials*. 2020; 21(1):1-17.
29. Newman E. and Kaloupek D.G. The risks and benefits of participating in trauma-focused research studies. Journal of Traumatic Stress: *Official Publication of The International Society for Traumatic Stress Studies*. 2004; 17(5):383-94.
30. Wilkinson A. Protocol transplant biopsies: are they really needed? *Clinical Journal of the American Society of Nephrology*. 2006; 1(1):130-7.
31. Khoja S.S., Khoja S.S., Khoja F.S., Khoja S.S. and Pirani N.A. A Review on Quality Agreement requirement in Pharmaceuticals by Regulatory Authority in Compliance to cGMP Guidelines. *PharmaTutor*. 2017; 5(5):24-8.
32. Jefford M. and Moore R. Improvement of informed consent and the quality of consent documents. *The lancet oncology*. 2008; 9(5):485-93.
33. Chowdary B. and George D. Improvement of manufacturing operations at a pharmaceutical company. *Journal of manufacturing Technology Management*. 2011; 23(1):56-75. https: //doi.org/10.1108/17410381211196285
34. Doolani S., Wessels C., Kanal V., Sevastopoulos C., Jaiswal A., Nambiappan H., et al. a review of extended reality (xr) technologies for manufacturing training. *Technologies*. 2020; 8(4):77. https: //doi.org/10.3390/technologies8040077

مراجعة شاملة لممارسات التوثيق في صناعة الأدوية

ثانوجا ر. [1]، ك. فينود كومار[2]، بيريدي ديفيا سري[2]، كارثيك س. [2]، ت. ريشما [1]*

[1] قسم ضمان جودة الأدوية، معهد راغافيندرا للتعليم والبحث الصيدلاني، كيه آر بالي كورس، تشييدو، أنانتابور، أندرا براديش، الهند.

[2] قسم تحليل الأدوية، معهد راغافيندرا للتعليم والبحث الصيدلاني، كيه آر بالي كورس، تشييدو، أنانتابور، أندرا براديش، الهند.

ملخص

تسلط هذه المراجعة الضوء على الدور الحاسم للتوثيق في صناعة الأدوية، وهي صناعة تتطلب التحكم في الجودة والامتثال للوائح بشكل دقيق. يشكل التوثيق، بدءًا من إجراءات التشغيل القياسية وصولًا إلى بروتوكولات التحقق، أساس أنظمة ضمان الجودة من خلال تشكيل وتحسين عمليات التصنيع. نستعرض أهميته في الحفاظ على جودة المنتج وسلامة المرضى وتلبية المتطلبات التنظيمية، خاصة في عمليات التفتيش من قبل إدارة الغذاء والدواء الأمريكية والوكالة الأوروبية للأدوية. بالإضافة إلى ذلك، نستعرض التحول الرقمي في ممارسات التوثيق، بما في ذلك تقديم السجلات الإلكترونية للدفعات والتحديات والفرص المرتبطة بها. يؤكد البحث على ضرورة التوثيق المتين والدقيق وفي الوقت المناسب، مشددًا على أنه التزام بجودة المنتج وسلامة المرضى والصحة العامة.

الكلمات الدالة: التوثيق، بروتوكولات التحقق، إجراءات التشغيل القياسية، إدارة الغذاء والدواء، صناعة الأدوية.

* ***المؤلف المراسل: ت. ريشما***

shaikreshmat614@gmail.com

تاريخ استلام البحث 2023/07/11 وتاريخ قبوله للنشر 2024/07/18.

الناشر

الجامعة الأردنية
عمادة البحث العلمي
عمان 11942 الأردن
فاكس: 5300815 6 00962

رقم الإيداع لدى دائرة المكتبة الوطنية
(2008/23.3/د)

عمادة البحث العلمي

جميع الحقوق محفوظة، فلا يسمح بإعادة طباعة هذه المادة أو النقل منها أو تخزينها، سواء كان ذلك عن طريق النسخ أو التصوير أو التسجيل أو غيره، وبأية وسيلة كانت: إلكترونية، أو ميكانيكية، إلا بإذن خطي من الناشر نفسه.

المجلة الأردنية في العلوم الصيدلانية

رئيس هيئة التحرير

الأستاذ الدكتور ابراهيم العبادي

أعضاء هيئة التحرير

الأستاذ الدكتور يوسف محمد الحياري — **الأستاذ الدكتور طارق لويس المقطش**

الأستاذ الدكتور معتصم عبد اللطيف الغزاوي — **الأستاذ الدكتورة فيصل توفيق العكايلة**

الأستاذ الدكتور وائل أحمد أبو دية — **الأستاذ الدكتورة ريما أبو خلف**

هيئة المستشارين

Prof. Zoltán Kaló
Center for Health Technology Assessment, Semmelweis University, Hungary
Prof. Ahmad Agil Abdalla
Biomedical Institute Research Center, Granada University, Granada, Spain
Prof. Nathorn (Nui) Chaiyakunapruk
University of Utah, USA
Prof. Ryan F. Donnelly
Chair in Pharmaceutical Technology, Queen's University Belfast, UK
Prof. Samir Ahid
Mohammed VI University of Health Sciences, Casablanca, Morocco
Prof. Udo Bakowsky
Philipps University Marburg, Marburg, Germany
Prof. Ayman F. El-Kattan
Executive Director, IFM Management Inc., Boston MA, USA
Prof. Paul Anthony McCarron
Head of School of Pharmacy and Pharmaceutical Sciences, University of Ulster, UK
Prof. Khalid Z Matalka
Matalka's Scientific Writing, Lexington, MA, USA
Prof. Habil. Wolfgang Weigand
Institute for Inorganic Chemistry and Analytical Chemistry, Friedrich Schiller University Jena, Germany
Prof. Ashraf Mostafa Abadi
Head, Pharmaceutical Chemistry Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, Egypt
Prof. Juan Manuel Irache Garreta
Universidad de Navarra, Pamplona, Madrid, Comunidad de, Spain
Prof. Ahmad Telfah
Leibniz Institut für Analytische Wissenschaften, ISAS Bunsen-Kirchhoff Str, German
Prof. Ali Qaisi
Faculty of Pharmacy, The University of Jordan, Amman, Jordan
Prof. Alsayed Alarabi Sallam
Al Taqadom Pharmaceuticals, Amman, Jordan
Prof. Karem Hasan Alzoubi
Faculty of Pharmacy, Jordan University of Science and Technology, Amman, Jordan
Prof. Yasser Bustanji
Faculty of Pharmacy, The University of Jordan, Amman, Jordan
Prof. Mayyas Al Remawi
Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan
Prof. Talal Ahmad Aburjai
Faculty of Pharmacy, The University of Jordan, Amman, Jordan
Prof. Qosay Ali Al-Balas
College of Pharmacy, Jordan University of Science & Technology, Irbid, Jordan

أمانة السر
سناء الدغيلي

تحرير اللغة الإنجليزية
لمى خليفة

الإخراج
نعيمة مفيد الصراوي

تعريف بالمجلة الأردنية في العلوم الصيدلانية

تأسست المجلة الأردنية في العلوم الصيدلانية بقرار لجنة البحث العلمي/ وزارة التعليم العالي والبحث العلمي رقم 367/2/10 تاريخ 2007/1/11 بشأن إصدار "**المجلة الأردنية في العلوم الصيدلانية**" ضمن إصدارات المجلات الأردنية الوطنية، وهي مجلة علمية عالمية متخصصة ومحكمة، وتصدر بدعم من صندوق دعم البحث العلمي والجامعة الأردنية تعنى بنشر البحوث العلمية الأصيلة المقدمة إليها للنشر في كافة مجالات العلوم الصيدلانية والعلوم الأخرى المرتبطة بها. وتصدر عن عمادة البحث العلمي وضمان الجودة في الجامعة الأردنية باسم الجامعات الأردنية كافة، خدمة للمتخصصين والباحثين والمهتمين في هذه المجالات من داخل الأردن وخارجه. وهي مجلة تصدر أربع مرات في العام أعتبارا من 2021، ومواعيد صدورها (آذار وحزيران وأيلول وكانون أول) من كل عام.

وباسمي وباسم أعضاء هيئة التحرير نود أن نشكر الزملاء الذين أسهموا بإرسال أبحاثهم إلى مجلتنا وتمكنا من إخراج العدد الأول. ونأمل من جميع الزملاء بإرسال ملاحظاتهم الإيجابية إلينا لنتمكن من النهوض بمجلتكم بالشكل الذي يليق بها.

وهذه دعوة إلى كافة الزملاء لإرسال اسهاماتهم العلمية من الأبحاث الأصيلة إلى عنوان المجلة.

والله ولي التوفيق

رئيس هيئة التحرير
أ.د. إبراهيم العبادي
قسم الصيدلة الحيوية والسريرية
كلية الصيدلة– الجامعة الأردنية
عمان 11942–الأردن